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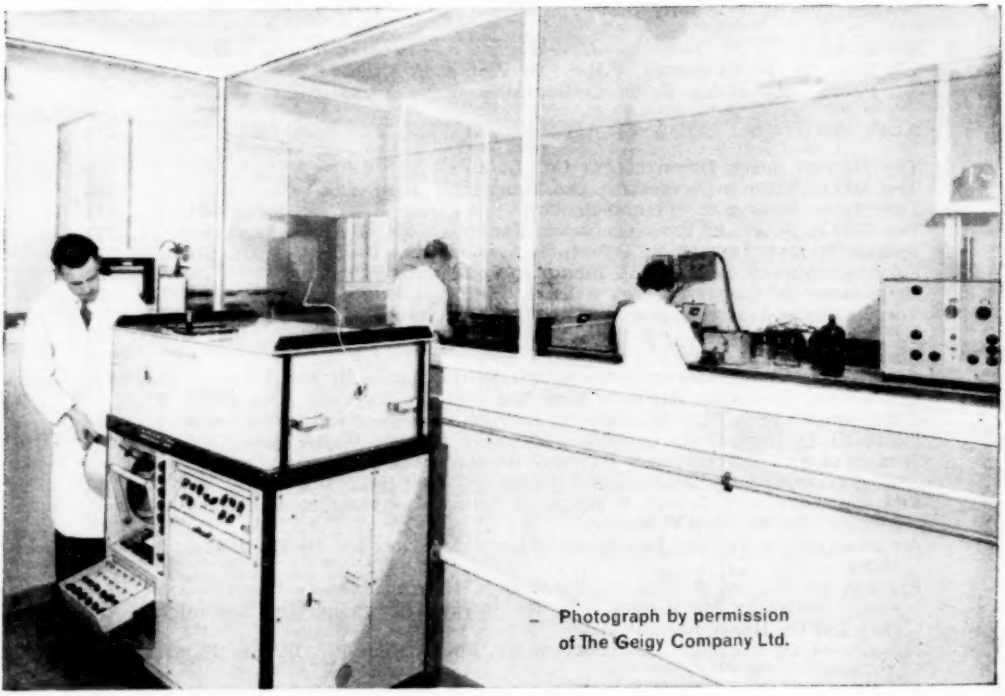

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FINANCING GOVERNMENT RESEARCH IN GREAT BRITAIN

IN the main, the report of the Council for Scientific and Industrial Research for 1960 is disappointing. The year has indeed been one of steady expansion, the pace and character of which have made it necessary, as Lord Hailsham points out in his introduction, to change the method of determining the Department's financial resources, a summary of which is given on p. 230 of this issue. The description of the change of methods is one of the most important features of the report; nevertheless, the report does not provide the information that might be expected, in view of the growing interest now being taken in the work of the Department, both in Parliament and elsewhere, on its contribution to the organization of Britain's research effort and the effective co-ordination of that effort. There is indeed a long and interesting section on the Department's relations with the universities and colleges of technology in Britain, dealing with the system of research grants and training awards; but a separate report is to be published surveying developments since 1956, emerging problems and trends in this field. Accordingly, that aspect of the Department's work is more appropriately considered when the promised report appears, just as the research associations and their work are now best discussed in the context of the separate report, *Research in Industry*.

Likewise, although no further information is given on the work of the Department's Intelligence Division, we are promised that the 1961 report of the Council will review at length the development of information services, and it is to be hoped that this report at least will supply the long overdue information on that part of the work of the Department. The Council, too, has been much concerned with the organization and maintenance of scientific representation, a function which the Department undertakes for all the Government departments concerned, and in which, in future, it may well be particularly concerned with the work of the new Department of Technical Co-operation. Here, the Council already recognizes from post-war experience that the true functions of scientific representatives grow naturally out of the local conditions in which they operate. The increasing importance of scientific questions in the political and commercial relations of the United Kingdom abroad has already made advice to ambassadors and other official representatives of the United Kingdom a major task of the scientific attachés. It is also increasingly important to promote good contacts between scientific organizations in Britain and corresponding bodies overseas, and the Government's own official scientific advisers continued to supply what is probably the most emphatic evidence of the consequences of the refusal to establish a Science Centre in London. Even now, there is little indication that official circles appreciate the extent to which the new National Lending and Reference Libraries of Science and Technology will, when fully operative, fall short of what such a Centre could have achieved in assisting scientific attachés in their task of securing an adequate supply of scientific and technical information from Britain.

The Council, however, recognizes that it is now necessary to consider extending the network of scientific representation so as to include not only countries that are scientifically and technologically advanced but also those that are at an earlier stage of development. Its decision that the time has come for a systematic assessment of the Government's needs in all areas which are not fully covered by the present network is welcome, as is the statement that the Council is also striving to ensure that the existing representatives have sufficient staff at their disposal to handle this increasing flow of important work. The Council has already embarked on a world-wide survey of the position in consultation with the Foreign Office, the Commonwealth Relations Office and the Board of Trade.

In view of what the Council itself writes in this connexion in the present report, one might have expected that the Colonial Office and the Department of Technical Co-operation would also be associated with this inquiry. Thus, the Department has been responsible since 1959 for the Tropical Products Institute, and this has led to a growing association of the Department of Scientific and Industrial Research with the needs of the dependent and emergent territories. The section of the report in which research into tropical problems is discussed refers to a steady demand from the less-developed countries for visiting experts, and they are continually looking for help in achieving a more effective utilization of their resources. The more advanced countries, on the other hand, request help with specific research problems or in finding new uses for over-produced commodities. Again, the report refers to investigations of fairly general interest, such as the possible physiological importance of tropical plants, and also the utilization of solar energy, which if it becomes economic could greatly benefit tropical and sub-tropical countries deficient in other sources of energy.

It is not, of course, the Tropical Products Institute alone which is concerned with studies of interest to the dependent territories, in the tropics and elsewhere. The Road Research Laboratory, the Building Research Station and the Forest Products Research Laboratory have long given assistance to such territories in particular fields. The Hydraulics Research Station undertakes, as part of its normal service to users, model studies and field investigations of problems arising overseas, and the territories concerned with the Station's present range of projects include Aden, Hong Kong, Iran, Mauritius, Nigeria

and East and West Pakistan; while during the year a number of its staff visited both Ghana and Nigeria to advise on the need for, and possible scope of, specialist laboratories there.

These developments, however, raise more than the questions of liaison on which the present report focuses attention. They involve questions of co-ordination and allocation of resources in which the Overseas Research Council at least is concerned, and the Department of Technical Co-operation may well be in future. There is no indication in the report that this is appreciated; but the matter is one which the Minister for Science might well examine as a matter of urgency. Some of the most urgently needed research could well be in territories least able at present to finance such investigations, and there appears to be *prima facie* a danger that the Department's disposition to insist on payment by the beneficiary may result in important investigations not being initiated. A clear-cut recognition of responsibilities and allocation of resources as between the Overseas Research Council and the Council for Scientific and Industrial Research should go far to eliminate this risk.

For the rest, the report shows that the Council is manifestly seeking to ensure, by its allocation of resources, that the scientific effort of Great Britain is balanced in relation to national needs. It regards the assessment of the balance of research in the universities and colleges of technology as fundamental, because in each subject strong and effective research departments are needed to attract good staff and students and to supply the nation with well-trained research workers. It is anxious to secure balance in the Department's own research programme and many of the changes introduced in recent years are for that purpose. In future, international action is likely to contribute much to the achievement of balance, and the Council recognizes that it must be prepared to play its part in co-operative projects.

In particular, the Council re-emphasizes the importance of surveying research needs in particular subjects and industries, as has already been done, for example, by its Economic Committee in respect of the British machine tool industry, and the ship-building and marine engineering industries. The Committee appointed under Sir Arthur Pugsley's chairmanship to review the need for research on the fatigue of engineering structures made of metal has also reported, and the Council has accepted its report. The main recommendation is that a co-ordinated programme of research should be undertaken in specified fields relating to structural materials used under fatigue conditions, particularly materials in structural sizes and joint assemblies, whether welded, riveted, or friction-grip bolted. Other recommendations are concerned with the co-ordination of this research and the provision of design information, based on the results obtained, to practising structural engineers.

The Department is discussing the implementation of these recommendations with the Institution of Civil Engineers and other professional organizations, but meanwhile the Council has decided to make the report immediately available. The Council has also continued to examine various proposals and applications for support and development work in industry, and it hopes soon to complete a number of preliminary studies of such projects, particularly costly ones. These are all activities which clearly fall well within the scope of the Council, which, it should be remembered, unlike the Advisory Council on Scientific Policy, is an executive body, and as such is an important tool of that Council for carrying out its policy.

Nevertheless, while the steady attrition of the annual reports of the Department of Scientific and Industrial Research tends to rob them of interest, which shifts to those of the Advisory Council on Scientific Policy, there are passages in the present report which suggest the reflexion that the Council sometimes trespasses on the functions of the latter body, while failing to supply the information for a sound judgment as to the wisdom of the policy it advocates. Comment on the statement that the Council has found insufficient research effort in fields of science such as biophysics, geophysics, meteorology and oceanography, which lie between the main disciplines, could be premature in view of the promised report on the relations between the Department and the universities, and in any event the Council is obviously seeking to implement a recommendation of the Advisory Council on Scientific Policy. Indeed, it is the findings reported to the Advisory Council last year that are now guiding the Council's own policy.

The main interest of the present report, however, lies, as already stated, in the description of the new method of allocating the Department's resources. On its appointment, the Council believed that the inherited system of determining the man-power and finances available for the Department's work in agreement with the Treasury for five years at a time—subject to the annual approval of estimates by Parliament—would permit the Department's activities to be planned in advance with a reasonable assurance that, so long as could be foreseen, they would not suffer a financial cut. Rapid changes in the scientific and technological scene and the increasing cost of tools of research have, however, since made it almost impossible to forecast with any accuracy the nation's requirements as long as five years ahead; and two years ago, at the launching of the Department's second five-year plan, the Council expressed the view that it was possible to estimate the needs of research fairly accurately two years ahead but only provisionally five years in advance.

These doubts have been confirmed by events. In last year's report, the Council referred to the unexpectedly rapid growth of applications for research grants from universities and colleges of technology. This year it points out that the accelerated expansion of scientific departments in the universities must be matched by an increase in awards for postgraduate training, if it is to yield its full benefit. The Depart-

ment is providing almost £300,000 for a space research programme, which only became possible after the five-year plan was prepared, and increasing use of the results of research in the conduct of Government business in itself leads to unforeseen demands on some of the Department's own research stations. Even more unpredictable are the requirements for very expensive pieces of scientific equipment, such as radio telescopes and particle accelerators.

Earlier this year, it thus became clear to the Council that adherence to the budget of the five-year plan would mean that much that should be done would be left undone, and that a more flexible financial instrument was needed. The new experimental scheme, which has been agreed with the Treasury, while providing reasonable assurance of future funds, is regarded as more adaptable to rapid and unpredictable change. Under it the Council will attempt to forecast its needs for five years in advance and will agree with the Treasury, subject to approval by Parliament: (1) the expenditure for the following year; (2) reasonably firm estimates for the two succeeding years (from which major departures will be made only if there are important changes in the scientific or financial situation); and (3) less-definite forecasts for the fourth and fifth years.

This system means that each year the Council will revise the estimates and bring them forward one year, so that they gradually become firmer. If the scheme works satisfactorily, the Council should be able to plan each year's expenditure alongside a fairly clear indication of likely expenditure in the years immediately following, and with some regard to longer-term plans. Whether this flexibility will be achieved at the cost of some decrease in security for research projects remains to be seen, but it would seem to be important, if confidence is not to be undermined, that the Department's annual budget should be large enough to provide a reasonable margin for adjustments.

The Council's understanding with the Treasury is that subject to the approval of Parliament the sums made available for the Department's work in what would have been the last three years of the second five-year plan will be more than 20 per cent above the original provision in the plan. Whether these provisions will give a sufficient margin of flexibility can scarcely be judged on the information disclosed in this report. What can be said is that, if the Council's annual report is to retain its importance and value, the legitimate segregation of detailed information to reports on particular phases of the Department's activities should not be carried to a point which hinders any general appraisal of the work of the Department as a whole. With Parliament taking a steadily increasing and healthy interest in the Department's work, it is the more essential that Parliament and public should be given ample information on which to judge the value of the work already being done, the needs and opportunities that are still not met and the way in which the Department's work impinges on that of other bodies contributing to the nation's research effort.

A. T. GREEN AND THE BRITISH CERAMIC RESEARCH ASSOCIATION

The A. T. Green Book

Dedicated to Arnold Trevor Green. By his colleagues of the British Ceramic Research Association. Pp. x+309. (Penkhull, Stoke-on-Trent : British Ceramic Research Association, 1959.) n.p.

DR. A. T. GREEN, who had been director of research of the British Ceramic Research Association from its formation in 1948, retired from this post at the end of 1959. His colleagues in the Research Association have paid him a worthy tribute by publishing this collection of original papers. In doing so, they have not only recorded their feelings of affection and respect for Dr. Green but also have shown what a lively and successful example of a research association the British Ceramic Research Association is.

The first chapter describes the development of the British Pottery Research Association and the British Refractories Research Association, the two bodies which were amalgamated in 1947 to form the present British Ceramic Research Association. The second chapter deals with the growth of the research programme of the present Association and traces the continuity and development of the programmes from the two pre-amalgamation bodies. Green was appointed director of the British Refractories Research Association in 1937. He had, for seventeen years, been associated closely with the first director, Dr. J. T. Mellor, in the development from the small beginnings in 1917. It is interesting to note that in 1933 Green had sponsored work in the Refractories Department of the University of Sheffield on the use of magnesia as a refractory.

The British Potteries Research Association, although beginning its activities much later than the Refractories Research Association, started off in much more favourable financial circumstances, and in 1938 it purchased "Beechfield", a large house with extensive grounds at Penkhull in which stand the present laboratories of the Ceramic Research Association.

The success of Dr. Green's work is aptly summarized by A. E. Dodd at the end of his chapter on some features of the work of the Research Association, as follows: "At no stage in the forty years covered has it been possible to point to a specific experiment and say 'This revolutionized ceramics'. Yet this has been the period during which 'pot-banks' and 'brick-yards' have, for the most part, become modern factories. This same forty years is the period—the lifetime—of service given to the industry by Dr. A. T. Green".

But this quotation does not quite express the extraordinary facility with which Green had persuaded gradually an old-fashioned industry, divided into many small independent firms, to support and use the results of the laboratory investigations. Not only did Green do this, but also he always maintained the background and interest in fundamental scientific investigations, and in the period before his retirement he made special efforts to convince the industry that in the future they must look for far more long-term work from the Research Association than they had in the past. This present volume shows how this new programme has taken shape.

XUM

In all there are 27 separate papers in the volume, and it is impossible to comment in any detail on all of them; many of them are statements of the present position of work in hand. Of these the first technical paper on thermal shock in furnace structures is a good example. The experimental observations are clearly stated, and the lines along which understanding has to be sought are carefully set out; no attempt is made to present a final answer. The experimental results are quite fascinating; the pattern of the fracture in refractory bricks subjected to a temperature gradient is shown to be very largely associated with the size of the brick, the 3-in. brick cracking parallel to the hot face, the 6-in. brick cracking perpendicular to it and the 9-in. brick cracking in two places perpendicular to the heated face. It is shown that the most probable explanation lies in the distortion of the sides, and it is made quite clear that the semi-infinite slab approximation would not be appropriate in considering this problem.

The paper on the constitution of bone china contains a very interesting historical survey of the developments in the understanding of the nature of this material and the function of the raw material components; the problem does not appear to be solved completely.

The newer items that are coming into the programme are well exemplified by the article on special ceramics in which the work in progress in the Association using titanates and non-oxide ceramics such as boron nitride is described. This is a good example of A. T. Green looking ahead and taking steps to influence the direction of his research programme. Work on sintered oxides started in the laboratories in 1953, and in 1955 he accepted a Government contract for work on new dielectric materials. These programmes have been characterized by the fundamental way of viewing the problem.

The more homely aspects of the work in the Association are typified by the chapter on glass colours and the effect of the newer detergents on the durability of these colours. Other papers deal with strain in wall tile glazes, the firing of intermittent kilns, extrusion research, rheology and ceramic casting-slip control, chrome-magnesite refractories, to mention only a few. In all, there are twenty-six contributions in this well-deserved tribute to Dr. Green.

R. W. Douglas

THE WEST RIDING OF YORKSHIRE

The Place-Names of the West Riding of Yorkshire
By A. H. Smith. Part 1: Lower and Upper Strafforth and Staincross Wapentakes. Pp. xi+346. Part 2: Osgoldeross and Agbrigg Wapentakes. Pp. xi+321. Part 3: Morley Wapentake. Pp. xiii+278. (English Place-Name Society, Volumes 30, 31 and 32.) (Cambridge: At the University Press, 1961.) 35*s*. net each volume.

SINCE its foundation in 1924, the English Place-Name Society has produced 32 volumes dealing with 21 counties. The present three volumes form the first instalment of a total of eight concerned with the West Riding, and they cover almost all the county to the south of the Aire. The next three will cover the rest of the county; the seventh volume will be devoted to the interpretation—linguistic and historical—of the material thus assembled; and the eighth will consist of a general index to the place-names of Yorkshire as a whole. We must, therefore wait for a discussion of the detailed evidence provided by this instalment; but, in the meantime, we can see what these volumes promise for the future and how they compare with the volumes that have already appeared for other counties.

Over the years, the Place-Name Survey has come to include an increasing number of minor names and field names, and this tendency is especially evident in these West Riding volumes. Many of the lesser names have no record earlier than the Tithe Awards of the mid-nineteenth century; but there is also a large number of names with forms from as early as the thirteenth century and even from the twelfth. All these names, together with the street-names of the larger settlements, are given a good deal of space in the present volumes. Complicated changes in the parish boundaries of the West Riding have involved many difficulties in the presentation of the material, but we are guided skilfully through the complexities of boundary changes. What is more, the area is highly industrialized, and, from beneath the modern built-up areas (with so many modern names), Prof. Smith has, in effect, disinterred an older landscape. The clearing of the wood, the draining of the marsh and the progress of rural settlement were activities that have left memorials in the place-names we use, often unthinkingly, to-day. Embedded in the detailed evidence there are many fascinating thumb-nail sketches of the history of individual names. The discussion of the name Halifax, for example, starts off with a review of early fanciful interpretations, and then proceeds with a piece of detective-like deduction. Another interesting sketch is that of the name Pontefract, which replaced the earlier Tateshale, now represented by the adjacent parish of Tanshelf.

This is the largest and most formidable county survey of place-names yet undertaken. The area is considerable; but, fortunately, the material is plentiful. These first three volumes whet our appetite for Volume 7 and for some general conclusions. The bulk of the names are Anglo-Saxon, but there is a strong Scandinavian element among them, and we look forward to a picture of the relative importance of English and Scandinavian names in different parts of the Riding. An idea of the bearing of this evidence on Yorkshire dialects will also be welcome; through the West Riding ran the boundary between Mercians and Northumbrians. We also look forward to knowing more about the West Riding Celtic names, not very numerous, but of especial interest because the Britons maintained the independent kingdom of Elmet here until well into the seventh century. Such parish-names as Monk Bretton, West Bretton and Walton suggest their own story. To the south, outside what may have been Elmet, there is Celtic evidence in the parish-names of Ecclesall and Ecclesfield near Sheffield, and in that of Wales not far to the east. In the north, just beyond the area dealt with in the present volumes, the name of Leeds is also Celtic in origin. Another category of names is that associated with Norman influence; the names are not many, but they give a feudal and French flavour to parts of the map of the West Riding, for example Hooton Pagnell and Laughton en le Morthen.

Two previous studies in the Survey (also by Prof. Smith) have dealt with the North Riding and the East Riding. The completion of the West Riding volumes will bring to an end a most ambitious

undertaking. The completed survey of the place-names of the whole of Yorkshire will carry our knowledge of the evolution of the life and landscape of England a most important stage forward. At a different level of comprehension and enjoyment, it is pleasant to turn over the pages and see the rich and curious variety that characterizes English place-names. Parish names such as Cridling Stubbs, Purston Jaglin and Thorpe Salvin cannot fail to excite our curiosity. These three volumes are not only for scholarly reference but also for delightful browsing.

H. C. DARBY

SCIENTIFIC ATTITUDE TO MANAGEMENT

Management Sciences, Models and Techniques

Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris, 7–11 September, 1959. Edited by Prof. C. West and Prof. Michel Verhulst. Vol. 1: Pp. xxix+602. Vol. 2: Pp. xiv+511. (London and New York: Pergamon Press, 1960.) 200*s*. net per set.

ESTABLISHED six years ago, the Institute of Management Sciences has made much more progress than even its enthusiastic founder members had cause to hope for. This is reflected in the *Proceedings of the Sixth International Meeting*, which was held in Paris during September 7–11, 1959, and which run to a weighty two volumes of concentrated report.

The meeting was supported by about a thousand participants from all parts of the world. Not unexpectedly, many of those attending came from the United States, and, again not unexpectedly in view of Britain's relative backwardness in management education, there were comparatively few British representatives. At present it would appear that other European countries and countries in the Commonwealth are showing greater interest in the scientific approach to problems of management than is the case in Britain.

Six years ago the complexity of management problems made it doubtful whether the scientist could make a noticeable contribution over a short period of time. During those six years the number of scientific personnel entering the management field has increased significantly, and there has been a corresponding if, as yet, limited advance along a number of sectors. Techniques like simulation, linear programming and the theory of decision-making have been applied to management tasks, sometimes with success verging on the phenomenal. Attention has also been paid to problem areas in production and inventory control, as well as to the place and performance of research and development departments.

These and related topics were all considered at the Paris meeting. Prof. Myron Gordon, of the Massachusetts Institute of Technology, showed that even the optimum amount of profit which a business should distribute can be analytically determined. Contributions from Norway and the Argentine gave examples of how little-developed industrial countries are using tools like operations research to stimulate their economies. From Britain, Prof. R. W. Revans presented a case-study to show how our hospital services need scientific help in their management, while F. de P. Hanika, now of Churchill College, Cambridge, presented a thoughtful paper describing the role of management sciences in training and education for management; his shrewd admixture of theory and practice should appeal to managing directors and teachers of management subjects in university or technical college.

Of the papers presented, however, a large proportion was directed to the area of business games. During the past few years there has been rapid progress in the development of analytical descriptions of entire business enterprises. These games are proving increasingly useful in management training as well as for research in investigating group interactions in industry and commerce; participants were able to sample games which had been developed and played in the United States, France, Denmark and Great Britain, and *Management Sciences, Models and Techniques* contains detailed descriptions of the game as well as comments from the players.

The two volumes make a noteworthy contribution to the application of science in management thinking and, by inference, indicate how ill-equipped many practising managers are to meet the challenge of the next decade. The need for a good basic education with more than a modicum of mathematics shines like a beacon on every page of this not inconsiderable book. So does the need for management scientists to eschew that extrapolative jargon which is already threatening to choke clear thinking and make them intelligible only to one another.

For such an important work the book is unevenly produced. Although intended for English-speaking readers, many outstanding American contributions are presented only as the briefest of abstracts; quite a number of contributions in French are set out in expansive detail, including the extraneous. It would appear that, for speed and economy, conference papers issued to participants have been printed by a number of different firms; when brought together to form a substantial book the effect is patchy and does not make for easy reading. Full contributions in one language with abstracts in the other might lead to the desired economies and produce more satisfying proceedings.

T. H. HAWKINS

TROPICAL FLORAS IN THE TERTIARY IN SOUTHERN ENGLAND

The Lower Tertiary Floras of Southern England. I. Palaeocene Floras; London Clay Flora (Supplement). By Marjorie Elizabeth Jane Chandler. Pp. xi+354+Atlas of 34 plates. (London: British Museum (Natural History), 1961.) 200*s*. net.

A LARGE part of this book is a supplement to *The London Clay Flora* by Mrs. Clement Reid and Miss Chandler, published by the British Museum (Natural History) in 1933. In this joint work they gave a description fully illustrated with photographic reproductions of the fossil plants, mostly fruits and seeds, found in the London Clay, which is part of the Early Eocene of the south of England. They devised special methods of investigating the internal structure of these fossils, which are partially lignitized and partially pyritized and uncompressed. A great deal of information about their histology could be obtained and enabled close comparisons to be made with the corresponding organs of existing natural

orders of flowering plants. It was possible to assign most of them to natural orders and, in a few instances, to existing genera. 234 species were described in the original work of 1933 : most were assigned to new genera and species. Miss Chandler now adds a dozen new genera and more than a hundred new species to the list. There are, in all, more than 50 natural orders represented in the London Clay. The geographical distribution of these orders today suggested that the London Clay flora was that of a tropical rain forest with a similar floristic composition to that of the present Indo-Malayan region. The Tethys Sea at that time included the present Mediterranean and extended from the Indian Ocean to western Europe, and this flora spread along the adjacent land areas. Miss Chandler's later work fully supports these views.

But, in addition to supplementing our knowledge of the London Clay flora, Miss Chandler makes important additions to our knowledge of the other Palæocene floras of England.

The Second World War interrupted much of her work, but in the previous eight years and in the period after 1944 much collecting has been done and there are many interesting and useful data about new localities. In the present work under review we find a systematic treatment of the Early Tertiary floras of southern England from Thanet, Woolwich and Reading Beds of the Pre-Ypresian to the Ypresian with the Oldhaven, Blackheath Beds and the London Clay. The geological relationship of these beds and the separate basins of the London and Hampshire areas is clearly explained. Their floras all suggest tropical conditions. There is no evidence for supposing that a cool climate existed during the Palæocene.

The systematic descriptions of the floras and their species occupy about three-quarters of the volume and are treated in a similar manner to *The London Clay Flora* by Reid and Chandler. The volume of illustrations contains 34 plates, which include more than 1,030 excellent reproductions of photographs of fruits and seeds.

Miss Chandler is to be congratulated on a great achievement. The work is marked throughout by accuracy, and the descriptions are always clear and unambiguous.

JOHN WALTON

K. TERZAGHI: FOUNDER OF THE SCIENCE OF SOIL MECHANICS

From Theory to Practice in Mechanics Soil

Selections from the writings of Karl Terzaghi. With bibliography and contributions on his life and achievement prepared by L. Bjerrum, A. Casagrande, R. B. Peck and A. W. Skempton. Pp. viii+425. (New York and London: John Wiley and Sons, Inc., 1960.) 96*s*.

PROF. KARL TERZAGHI is one of the most interesting figures in the world of engineering science, and many believe him to be the greatest civil engineer of our day. Due to his pioneering research in Istanbul, leading to the publication of *Erdbaumechanik* in 1925, the study of the mechanical properties of soil was given a rational basis, and the science of soil mechanics, which is now studied by civil engineering students throughout the world, took its place among the other engineering disciplines.

Soil mechanics is itself divided into a number of specialities, for example the stability of earth dams, the design of pavements, the heaving of soil due to frost, etc., and it is astonishing how many of these various topics were first tackled systematically by Terzaghi, leaving it to others to develop along the lines he first indicated. This might have been deduced from his *Soil Mechanics in Engineering Practice* (1948); but it is brought out very clearly in this collection of his early papers, which trace in a fascinating way the development of soil mechanics, as we now know it, with the man. To the younger generation Terzaghi is already becoming a mythical figure. His James Forrest Lecture at the Institution of Civil Engineers in 1939 stirred their elders into a realization that soil mechanics was of vital concern to them; now, in this work, all may come closer into contact with him and learn from his directness and integrity.

A few of the collected papers published here would have been available to readers in a big city with a good library. But the *Proceedings of the First Soil Mechanics Conference* (1936) are somewhat rare, and many will welcome the opportunity of having Terzaghi's contributions to it on their own shelves. Most of the other papers will be quite new, however, and of these the professional reports are the most valuable. Apart from their interest they provide a model of good prose.

The book has two particularly valuable sections. One, contributed by Prof. Skempton, assesses the importance of the concept of effective stress, discovered by Terzaghi, from which so much of the theory of soil mechanics is derived. The other, by Prof. Casagrande, is a biography of Terzaghi, which fills in some of the detail of the image which surrounds him. As he approaches his eightieth year, we are grateful to those who made this collection and edited the work. Few men of this stature emerge in each generation.

KEVIN NASH

MELBOURNE SYMPOSIUM ON ANTARCTIC METEOROLOGY

Antarctic Meteorology

Proceedings of the Symposium held in Melbourne, February 1959. (Commonwealth of Australia: Bureau of Meteorology.) Pp. xviii+483. (London and New York: Pergamon Press, 1960. Published for and on behalf of the Director of Meteorology, Melbourne, Australia.) 105*s*. net.

NO criticism of the contributors to scientific symposia such as the one here presented would be implied by the comment that, in some cases, it may be doubted whether the papers are of such lasting value as to justify a hard-covered book selling at a high price. There are, after all, the appropriate scientific journals as media for publication. This particular symposium was, however, a special case, held under the official auspices of the Special Committee for the International Geophysical Year and the Special Committee for Antarctic Research shortly after the conclusion of the International Geophysical Year.

The symposium (*Nature*, **183**, 1503; 1959) was in fact a meeting of workers from the International Geophysical Year expeditions, then on their way home. In view of the fact that it was held so soon after the

observations had been made, and with so little time for digestion, the content appears remarkably good. As might be expected from the numbers involved in this major scientific onslaught on Antarctica, the problems tackled are extraordinarily diversified—one dare not say comprehensive: there is, for example, little about oceanography or the sub-antarctic sea ice, though there is some interesting material for considering the role of the stratosphere; and, of course, local effects could only be sampled.

The forty papers printed were distributed between sessions on (1) local effects, (2) synoptic analysis and forecasting, (3) synoptic influences on lower latitudes, (4) general atmospheric circulation, (5) snow and ice characteristics, (6) heat and mass exchanges, and (7) climatology. In some of these fields, including both synoptic meteorology and the antarctic stratosphere, the book provides by far the richest reference material so far in print; the synoptic examples are, of course, largely drawn from a period far too short to be fully representative, and some of the contributors (if given longer experience in antarctic meteorology) might with advantage have drawn more on comparisons with earlier published synoptic situations. The work of Meinardus, Simpson, Palmer and Ramage—to quote a few—seems under-represented in the references used.

Until recent years only one or two general reference books existed from which the meteorologist working in antarctic regions, or his colleague concerned with antarctic effects on the weather and climate of the southern hemisphere homelands, could obtain guidance. The present work will be a very valuable addition to the libraries which such workers can consult—though for this purpose a book should have an index. It seems likely to take its place, for several years to come, among the four or five most used works on antarctic meteorology.

H. H. Lamb

MATHEMATICS AND LANGUAGE

Type-Token Mathematics

A Textbook of Mathematical Linguistics. By Dr. Gustav Herdan. (Janua Linguarum, Series Maior, Vol. 4.) Pp. ii+448. (The Hague: Mouton and Co., 1960.) 54 guilders.

THE application of statistical methods to linguistic problems goes back at least as far as Malone's suggestion (in 1778) that the order of writing of Shakespeare's plays might be ascertained by such tests. In the past fifteen years it has gained additional interest from the developments of 'information theory' and projects for mechanical translation. Various methods have been evolved, and some quite novel statistical techniques discovered: but a laborious search of both statistical and linguistic publications is needed to discover what has been done. A book which attempts a survey of the whole of this field therefore deserves some welcome.

Type-Token Mathematics has much the same scope as Dr. Herdan's earlier book *Language as Choice and Chance* (1956). It is considerably more than a rewritten and enlarged version, as it contains both new ideas and new material; several chapters are reprints of recent papers by the author. Its material falls into two parts (closely commingled): mathematical and philosophical. The mathematical parts are vitiated, unfortunately, by the extreme shakiness of Dr. Herdan's statistical technique: the innocent linguist must be urgently warned not to adopt any procedure that he recommends without advice from a statistician. Two examples will suffice to put the statistician on his guard: it is not true that if we use a significance test at the 1 per cent level "we shall be wrong once in 100 times with our assumption of a real difference" (page 105); and it is not true that "deviations [from the mean] exceeding three times the standard deviation" are very unlikely to occur by chance (page 287) when we are considering a variate which has (approximately) a Poisson distribution with an expectation of less than one (as on page 290). In the latter passage, it is regrettable that Dr. Herdan gives unreliable formulæ for α (the expected frequency in a second sample of a word occurring a small number of times in the first) and its variance, as the techniques for making reasonably accurate estimates form one of the most interesting developments of statistics applied to language. Dr. Herdan nowhere mentions these techniques, although his bibliography includes the paper containing them (Good, 1953).

The philosophical portions of the book are occasionally stimulating, but more often irritatingly vague and confused. A fairly extensive bibliography is given; unfortunately it fails to include several of the references in the text, leaving the reader to search for, for example, "Oettinger, 1957", with no further clue. In view of the sub-title it should be added that the book is essentially an exposition of Dr. Herdan's own, generally controversial, views—a perfectly proper purpose for a book in general, but most inappropriate for a 'text-book'.

A reader whose knowledge of both statistics and information theory is sufficiently sound to save him from being misled or confused may find some interesting material and some interesting ideas in the book, but is unlikely to find it good value for money. The beginner in these fields must be, reluctantly, warned to keep off it.

G. H. Toulmin

MATHEMATICS AND QUEUEING

Mathematical Methods in the Theory of Queueing

By A. Y. Khintchine. Translated by D. M. Andrews and M. H. Quenouille. (Griffin's Statistical Monographs and Courses, No. 7.) Pp. 120. (London: Charles Griffin and Co., Ltd., 1960.) 32*s*.

THIS beautiful little book breathes reason and modesty from cover to cover. It is written somewhat in the style of the classical French texts of fifty and more years ago—a style which allowed sufficient space for an honest exposition, and was not cramped by the present-day fashion for condensation and the pressure of costs. The publisher and the translators are to be congratulated for introducing the work to the English-speaking world, and the book will be of undoubted value to every mathematician interested in the theory of queues. A particularly pleasing feature is the way in which the results are developed; the mathematics is 'done', not merely indicated, and nowhere does the author state a result and refer the reader elsewhere for the details. Thus there is a satisfying aspect of completion about the exposition.

Part 1 is devoted to the theory which depends only on the arrivals and not on the nature of the service-

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time distribution. A 'simple' stream is defined to be one which displays stationariness, absence of after-effects, and orderliness; that is, the probability of k arrivals in any interval of time depends only on the length of the interval, and not on any arrivals before the start of the interval, and there cannot be two or more arrivals at the same instant. The theory of the simple stream is developed, and then extended by relaxing one or more of the conditions just stated. In particular, some properties of the stream without orderliness are obtained, and streams with limited after-effects are investigated.

In Parts 2 and 3 problems of service are considered. In a queueing system with several lines of service (in parallel) a customer on arrival may find that there is no service immediately available. He may wait until service is available, or may leave the line without waiting. In the former case one speaks of a "system allowing delay", in the latter case of a "system with loss". It is perhaps worth pointing out that Chapter 11 (the last) presents the general theory of a single-line system with an arbitrary service-time distribution. The distribution function for the waiting time is found in principle by a fairly simple method. The contents of the chapter are taken from a paper by the author, published in 1932, and present material which will be new to many readers.

The theory of queues has undergone considerable development in recent years. Some mathematicians think the development has gone too far. Whether this is so or not the book under review will serve to show that the phenomenon of queueing represents another human experience which has bowed to the forces of applied mathematics; the concepts that have been built around this experience have proved to be of the right kind, and sufficient in number, for the mathematical development to go 'with a bang'. Anyone who is in doubt as to whether applied mathematics is a living subject will find it rewarding and reassuring to read this book.

L. S. Goddard

ARCHÆOLOGY OF OLD SMYRNA

The Annual of the British School at Athens
Nos. 53–54, 1958–1959. Pp. xii+312+74 plates. (London: The British School at Athens, 1960.) 105*s*. net.

With this number begins the serious publication of Old Smyrna, dug some ten years ago. Most of the articles on other subjects, to take these first, are also rather austere. Sinclair Hood and Piet de Jong describe a Minoan kitchen. Sinclair Hood, G. Huxley and N. Sandars a cemetery of the Late Minoan III Period, both near Knossos. Analysis of amber beads leaves their provenance uncertain. J. Leatham and Sinclair Hood describe some submarine work on sunken fish-ponds and harbours; work which, like the Mycenæan boat off Lycia, confirms that at present diving offers the bleakest prospects in archæology. R. Hope Simpson finds the rag of a Mycenæan settlement near Menidi, in Attica. J. M. C. Toynbee describes four interesting Roman portraits, dating from the Claudian to the Decian Period, in the Piræus Museum; and John Boardman describes a farmhouse inhabited about 450–350 B.C. at Pindakas in Chios, a site remarkable chiefly for a coin-hoard of about 350 B.C. Boardman has interesting things to say about the first-named 'magistrates' issues of Chios, and about her earliest bronze coins, which he would date about 375 B.C.

J. M. Cook introduces Old Smyrna in a topographical and historical résumé—a model of masterly brevity badly needed in this volume. The settlement at the north-east end of the Gulf goes back to Protogeometric times. "Aeolic" at first, it had become Ionic by about 800 B.C., which might agree with the story of its seizure by Colophon. The eighth century was the era of rounded houses. An earthquake about 700 B.C. destroyed the walls, which were not immediately replaced, while the new houses were rectangular. About 600 B.C., when a new wall had scarcely been finished, a new temple only begun, the Lydian Alyattes raised a siege-mound (still surviving, to the north-west) and took the city. After that, though quite densely inhabited by the fourth century, Old Smyrna never recovered its status, and was deserted for the present site before 300 B.C. J. K. Anderson, who describes the Corinthian pottery found, shows that Alyattes sacked the town well before the Early Corinthian style had run its course. Prof. Cook finds space for a mass of topographical information, subsidiary but never irrelevant.

John Boardman describes the Attic vases, some from good workshops. But the core of this instalment is an extremely painstaking hundred pages, by R. V. Nicholls, on the City Walls. The illustrations, through all their intricacies, maintain a high level of scientific exactitude and consistency; although even Mr. Nicholls grows weary in time of using all the symbols and *sigla* recommended by Sir Mortimer Wheeler, and finds (for example, in Figs. 7 and 10) that he can simplify with advantage. Somewhere in the text are many excellent points; for example, that the mound to the south-east of Gordium could also be a siege-mound, that the staked ditch of Iliad XII is more likely to be Iron Age than Bronze Age, that the step on the inner face of our Walls 2 and 3 might have supported a wall-plate to drain the walls' *chemi-de-ronde*, and that mud-bricks in the mid-seventh century changed to a size and shape more suitable for the new two-storey houses. It is, alas, typical that this last sensible conclusion should be mixed up with pages of metrological discussion (Nicholls believes, prematurely I feel, that only two constant lengths of foot existed and are known for the Classical Period.) These reach no firmer conclusions than one might expect, and lead by the way (p. 105) to such things as single bricks with dimensions on conflicting scales. Even Le Corbusier has not got to that. On important matters, like the arrival in the Greek World of the true arch, the citation of authorities seems most one-sided. For all its acuteness, the article is almost impossible to read. Page after page, one has to endure sentences like this: "The brick dimensions quoted on p. 101 are also of great interest in this regard, notwithstanding that it is not always possible to evaluate the different degrees of reliability with which they have been taken". Mr. Nicholls evidently knows his Herodotus and Homer. Yet, as stylists, they must mean nothing to him. Similarly, modern Athens has the most wonderful museums in the world, and the ugliest streets. Humanity, in its own home, has been sterilized.

Hugh Plommer

Genetica

By Prof. Enrique Sanchez-Monge y Parellada. Pp. xii+437. (Madrid: Enrique Sanchez-Monge y Parellada, Instituto Nacional de Investigaciones Agronomicas, 1961.) 380 pesetas.

THE present work by Prof. Sanchez-Monge is based on a previous book by him and Dr. Ramon Esteruelas published in 1952. The rapid development of genetics during the past few years has made rewriting necessary, though the objectives which animated the first work are unchanged. These were, states the preface, briefly to put into the Spanish language all the fundamental bases of modern genetics so that the reader may appreciate the importance of this discipline in pure and applied biology, medicine, psychology, agronomy, forestry, taxonomy, the improvement of plants and animals, microbiology and virology.

Such a book was highly necessary in Spain, where, it is alleged, outmoded ideas which should have been abandoned years ago still have a firm hold on the curriculum.

The present work differs in its general plan from the first one, principally in the change of title, and in the omission of two chapters on the improvement of plants and animals. It begins with an introductory chapter on reproduction, and then goes on to chapters on Mendelian heredity, qualitative and quantitative, the latter being preceded by a brief introduction to statistical analysis. It continues with an account of sex determination and sex-linked inheritance, types of evolutionary change, the role of cytoplasm in heredity, the genetics of populations and the physiology and nature of the gene. Then follows a chapter on genetical aspects of evolution, with special reference to mechanisms of speciation and to the species concept. Finally there are two chapters dealing with the genetics of micro-organisms and of man. Considering the wide field that the author has surveyed it is not surprising that treatment sometimes fails to achieve correct emphasis. For example, the chapter on human genetics contains no mention of the work of Penrose or Haldane, nor of the contributions of Harris in the domain of human biochemical genetics.

Five appendices contain: a short history of genetical discoveries, the international rules regarding symbols, a glossary of technical terms, a collection of problems and a list of recommended books. The book contains an admirably clear, concise and on the whole accurate account, in 437 pages, of present-day genetics, and is recommended to all students who read Spanish. It is well produced and illustrated and relatively cheap, though of somewhat inconvenient size. Each chapter contains a useful bibliography and there is a good index.

S. C. HARLAND

Proceedings of the Third World Orchid Conference

London, May 30–June 2, 1960. (Sponsored jointly by the American Orchid Society, The British Orchid Growers' Association and the Royal Horticultural Society.) Pp. 442+10 plates. (London: The Royal Horticultural Society, 1960.) 42*s*.; 6 dollars.

THE third World Orchid Conference, held in London at the beginning of June 1960, brought together more than five hundred persons, from thirty different countries, whose common interest was the study of the family Orchidaceae, albeit from many different points of view. Professional growers, amateurs and scientists were among those attending, and the numerous papers delivered, though with a distinct bias towards cultivation, covered a wide range of aspects of orchidology. This volume gives a general account of the programme and proceedings of the conference and includes all the papers delivered during its thirteen sessions.

The Conference was given an excellent send off by Dr. W. T. Stearn of the British Museum (Natural History), who entertained the members with a scintillating and highly humorous account of the development of orchidology from classical times up to the present day. Following this, each session dealt with some special aspect of the subject or with one of the more important groups of cultivated orchids. The former include such diverse subjects as culture methods for amateurs, the principles of orchid nutrition, the ecology of orchids, diseases and pests, aspects of the orchid trade, the classification of orchids, growing orchids in rooms, and orchids in botanical gardens. The papers on cultivated orchids deal with the genera *Cattleya*, *Vanda*, *Phalaenopsis*, *Cymbidium*, *Dendrobium*, *Cypripedium*, *Odontoglossum*, *Miltonia* and their close allies. These last-named papers include accounts of the latest methods of cultivation, progress in breeding and criteria used in judging. Altogether there are forty-eight papers and these taken together provide an adequate cross-section of the various activities which are being pursued to-day with respect to this remarkable and entrancing family.

The general format of the book is pleasing, the print being easy to read. There are ten excellent coloured plates of orchids which gained awards in the Royal Horticultural Society's Chelsea Show held just before the Conference and nearly thirty monochrome illustrations. In addition many of the papers contain illustrative text-figures. Altogether this is a book which everyone interested in orchids will require, containing as it does many of the latest ideas bearing on the cultivation, breeding and classification of these plants. V. S. SUMMERHAYES

Advances in Agronomy, Vol. 12

Edited by A. G. Norman. (Prepared under the auspices of the American Society of Agronomy.) Pp. xi+464. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12.50 dollars.

THE present volume fully maintains the standard set by recent issues of *Advances in Agronomy*. It contains eight reviews of United States work each of which is long, detailed, exhaustive and dull. Research papers are often summarized in single sentences and more-or-less contrary findings by different authors are recorded consecutively with serene impartiality and little or no critical comment.

Of the articles in this volume, that by Rich and Thomas on the clay fraction of soils is unusual in including many references to work done outside the United States and in dealing with recent work—real 'advances'. "Fertilizers in Forestry" (Stoeckeler and Arneman) also makes a good attempt at bringing together work done in many countries. Two papers on soya beans (81 pages in all) leave the reader with the impression that a great deal of research on this crop is proceeding, but that very little of it is likely to lead to increases in yield before 1980.

"Water Infiltration into Soils" (Parr and Bertrand) catalogues many fascinating pieces of apparatus and

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indicates the difficulties in measuring infiltration in the field. Fergus and Hollowell give a good historical account of red clover and research in progress on it; this paper includes a good account of husbandry in the field.

Of the rest, the paper on "Agronomy in the Southwest United States" seems misnamed—it describes the agriculture of the area; the paper on grass and legume seed-production and testing omits all reference to the inoculation of legume seed, and in a long section on diseases dismisses bacteria, viruses and nematodes in one sentence. G. V. Dyke

Flora of Peru

By J. Francis Macbride. (Botanical Series, Vol. 13, Part 5, No. 1.) Pp. 536+ii. (Chicago: Field Museum of Natural History, 1959.) 8.50 dollars.

MR. MACBRIDE continues his *Flora of Peru* with a volume devoted to the Araliaceae, Haloragaceae and a number of difficult gamopetalous families such as Ericaceae (including Vacciniaceae), Myrsinaceae, Ebenaceae, Gentianaceae, Apocynaceae and Convolvulaceae. The accounts of important genera such as *Buddleia* and *Strychnos* are also here. This work, as before, is a useful compilation as a basis for monographic work by specialists, and the author is an adept at pointing out the "interlocking characteristics" of the "species groups at present designated as genera" in some families. His numerous remarks in this vein force one to the conclusion that it is no good having 'specialists' in some of these families unless they have first-class brains and time for concentration on a wide survey over a long period. Mr. Macbride is old-fashioned enough to use the illegitimate name *Erythraea* for *Centaurium*, and he ignores the name *Gentianella* for a segregate genus covering the many Andine gentians, which he maintains in *Gentiana*. On p. 152, *Samolus Valerandi*, to which the South American *S. floribundus* Kunth is reduced, is unfortunately misprinted *S. Verlanderi*.

N. Y. Sandwith

The Atomic Problem

A Challenge to Physicists and Mathematicians. By Lancelot Law Whyte. Pp. 56. (London: George Allen and Unwin, Ltd., 1961.) 7*s*. 6*d*. net.

THE author calls for a drastic revision of the principles of physics in order to find answers to unsolved problems in that branch of science. In particular he wishes to retain the "principle of atomism" and to discard "linear co-ordinate systems, dimensional parameters, two-entity interactions and time-invariants". Many of his propositions could be interpreted in different ways, but there is an unambiguous demand for a theoretical deduction of the fine structure constant; there is also a clear request for including irreversibility in the basic laws of the new physics. While not attempting to substantiate his programme the author expresses the hope that others might follow his directives.

The fundamental principles of a science are due for revision if unsolved problems prove *insoluble*. In contemporary physics this situation does not arise: existing theories have neither spent their force nor are they tied to any rigid preservation of all their details. Current attitude to irreversibility belongs to the best secured domains of physics. The problem of the fine structure constant will attract more attention than at present if it acquires any direct experimental implications. Perhaps the author believes that theoreticians are a complacent lot; they still continue to interpret new discoveries in terms of old theories unless incompatibility is clearly recognized. It would not be wise to prophesy now which of the author's propositions will materialize in a future revision. R. Eisenschitz

An Introduction to Infinitely Many Variates

By Prof. Enders A. Robinson. (Griffin's Statistical Monographs and Courses, No. 6.) Pp. 132. (London: Charles Griffin and Co., Ltd., 1959.) 28*s*.

THE word 'Variates' in the title means 'random variables'. The first twenty pages give an account of probability theory as a normed measure over an abstract space. The next fifteen discuss limit theorems: followed by sixteen on Hilbert space and linear operators. These latter ideas are used in twenty pages on the spectral representation of stochastic processes and thirty pages on stationary processes. There is a large and useful collection of exercises. There is an extensive use of advanced mathematical ideas and notations, but practical problems are not entirely ignored (for example, section 7.9 on wavelet theory).

The monograph has been designed to give undergraduates an introduction to advanced techniques early in their statistical training. To my mind it is too condensed for this, and makes too many errors. Students at this level will not be familiar with the Radon-Nikodym theorem, and therefore will not know that it is stated wrongly on p. 13—nor the inversion theorem on p. 30—nor the definition of continuity on p. 19. But the book could be valuable for readers who can spot such mistakes and require a brief introduction to limit theorems and spectral analysis. The book would have been better had it been more carefully written and had it not been so curt, for the material is well organized. D. V. Lindley

Chemistry of Carbon Compounds

A Modern Comprehensive Treatment. Edited by Dr. E. H. Rodd. Vol. 4, Part C: Heterocyclic Compounds. Pp. xviii+1465–2201. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 130*s*.

WITH Volume 4 of this work the section on heterocyclic compounds is complete and the whole project draws near to its conclusion. This volume continues the systematic treatment of six-membered ring systems containing two heteroatoms, proceeds to those with more than two and hence to seven-membered and larger heterocycles. Separate chapters present excellent accounts of azine dyes, of purines, of nucleosides and nucleotides and of pteridines. About half the volume is devoted to a survey of alkaloids, largely written by A. R. Pinder, with contributions by K. W. Bentley and G. F. Smith. They and the other contributors are to be congratulated on a high order of achievement. The more familiar terrain and the outer reaches of the subject are charted with competence and lucidity that belie the magnitude of the tasks involved.

This is no mere cataloguing, but a genuine appreciation and appraisal of the state of these chemical topics, close to the date of publication. Once again a team of authors and a redoubtable, indefatigable editor have put the world of organic chemists in their debt. James D. Loudon

THE CROSS-ANTENNA OF THE PROPOSED BENELUX RADIO TELESCOPE

By Prof. W. N. CHRISTIANSEN* and Dr. J. A. HÖGBOM
Benelux Cross-Antenna Project, Leyden

SEVERAL years ago, C. L. Seeger of the Netherlands Foundation for Radio-astronomy proposed[1] that a very large radiotelescope of the Mills cross type[2] should be built in Europe for high-resolution studies in radio-astronomy. The main feature of this radio telescope was to be its resolving-power of one minute of arc at a wave-length suitable for observations of discrete radio sources and of the structure of nearby galaxies.

The dimensions of this antenna would be so large that it would be necessary to distribute radio-frequency amplifiers along the antenna in order to overcome a loss of sensitivity which would be introduced by severe attenuation in the transmission lines between antenna and receiver. It was suggested that these amplifiers could be made to serve an additional useful purpose, by using them in a way similar to that in the large grating-interferometer at Nançay in France[3]; the input of each amplifier would be connected to a small section of the antenna and the outputs of the amplifiers would be split into n separate channels. The output of each channel would then be correctly phased and combined with the outputs of one channel of each of the other amplifiers. The result of this is that each channel could be made to respond to radio emissions from a slightly different direction in the sky, so that with no loss in sensitivity the antenna would have n separately usable beams instead of one. By this means observing time can be reduced by a factor of n; the necessity for this is obvious when it is realized that with a

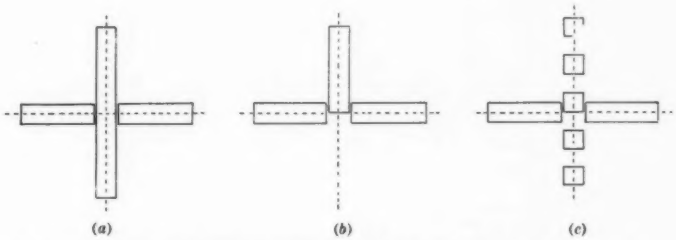

Fig. 1. Antennæ with similar beam-width. *a*, A Mills cross; *b*, a T-antenna; *c*, a displaced-centre grating cross

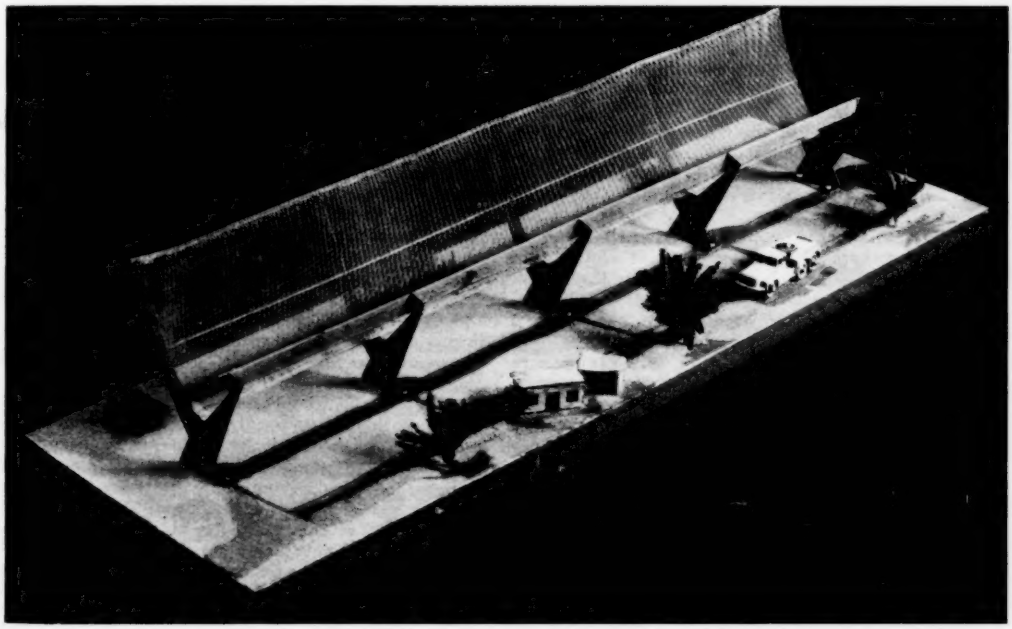

Fig. 2. A model of one section of the north–south array of the proposed Benelux cross. The dimensions are 15 m. × 85 m.

* On leave from the University of Sydney.

single 1′-arc beam, approximately 50 years would be needed to complete a survey of the sky.

As a result of this proposal, a committee with Netherlands and Belgian representation was set up under the chairmanship of Prof. J. H. Oort to investigate the possibility of building such an instrument. The committee is known as the Council of the Benelux Cross Antenna Project. A report was prepared[4] specifying the wave-length to be used (73 cm.), the size (600,000 m.²), the resolving power (1′ arc) and emphasizing the necessity of designing the antenna to have very low response outside the region of the principal response.

Funds for the design of the large Cross Antenna were provided jointly by the O.E.E.C. Committee on Scientific and Technical Personnel, the Netherlands Organization for Pure Research (Z.W.O.) and the Belgian Ministère de l'Instruction Publique.

Recently, we have prepared a report[5] in which a suitable design for the cross-antenna of the Benelux radio telescope is submitted; we shall describe its main features here, since some of these are novel and may be of use to those interested in the planning of other large radio telescopes.

The original Mills cross[2], which had a beam-width of 50′ arc, consisted of a north–south and an east–west array of parallel dipoles; the pencil-beam response of the antenna was produced at the intersection of the fan-shaped responses of the two arrays by a multiplication process first used by Ryle[6] in radio-interferometry. The angle of elevation of the principal response of the cross could be changed by readjusting the phases of currents transmitted to the receiver by the individual dipoles of the north–south array; this changed the angle of elevation of the fan beam of the north–south array and hence the direction in which this beam intersected the beam of the east–west array. The scanning of the sky in hour-angle was produced by the rotation of the Earth. One feature of this cross was that the 'steering' of the antenna response was effected without mechanical movement of any part of the antenna.

For an antenna which has an aperture (in square wave-lengths) three or four orders of magnitude greater than that of the first Mills cross, the use of simple fixed dipole arrays is impracticable, since the number of dipoles required is about 10^6–10^7 and the number of separately phased groups of dipoles is 10^4–10^5. The number of active elements in the antenna may be reduced by using parabolic reflecting surfaces; but these, except in special cases, must be capable of being tilted to allow the direction of the principal response of the cross-antenna to be varied.

In two of the large Mills crosses which are being built or planned, one in the U.S.S.R. and one in Australia[7], line arrays of dipoles are backed by cylindrical parabolic reflectors. For the east–west array these reflectors are capable of movement about the long axis of the array; in the north–south array the reflector is fixed, and the direction of the fan-beam response of the arm is varied by phase-changes in the dipole-array, situated in the line-focus of the parabolic reflector, in a similar fashion to that of the original Mills cross.

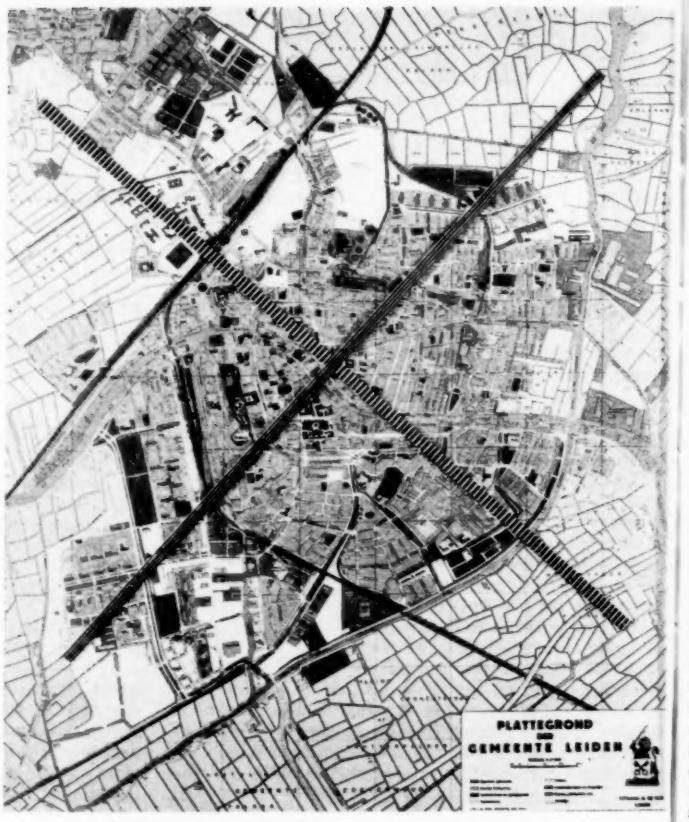

Fig. 3. Suggested arrangement for the 5,000 m. × 5,000 m. Benelux cross-antenna. The antenna is superimposed on a map of Leyden to provide a scale

For the projected Benelux cross, which would have dimensions several times greater than these two crosses, such an arrangement would involve the separate phase-adjustment of more than 10^4 closely spaced elements for each change in declination of the antenna response. We have tried, therefore, to find a solution to the problem that will involve the phase-adjustment of only a limited number of elements. If the north–south array is divided into sectors each of which can be tilted in declination, the number of elements requiring separate phase adjustment can be reduced by a factor of about 100; moreover, the phasing difficulties are considerably reduced because the coupling between the sections can be made negligible. The difficulty which is introduced by this arrangement, however, is that each element tends to 'shadow' its neighbour as the array directed away from the zenith. If we separate the elements to avoid this shadowing, then multiple antenna responses are found; these are similar to those of a grating interferometer[8], and for the purposes for which the Benelux cross is designed, highly

undesirable. We have found, however, that it is possible to overcome these difficulties.

It is known[9] that one of the four arms of a cross-antenna (Fig. 1*a*) can be removed without restricting the spatial harmonic response of the antenna, or in other words, without increasing the beam-width of the principal response of the antenna.

If, in such a T-antenna (Fig. 1*b*), we remove alternate sections of the asymmetrical arm, and place them on the opposite side of the antenna, as shown in Fig. 1*c*, then we have a cross-antenna in which one array is broken up into spaced sections. The unwanted grating-responses of this arm have been suppressed, however, by the displacement of its centre with respect to the centre of the continuous array. With this arrangement, we can now tilt the sections of the north–south array over a wide range of angles without having one section fall in the shadow of its neighbour.

It will be seen that the antenna design just described does not provide a complete solution to the problem, since the proportion of the north–south arm which is filled by the antenna elements changes with the angle of elevation. This, and non-uniformity of illumination of the elements of the north–south array, will produce the effect of a 'ripple' along the north–south array and unwanted secondary responses of the antenna will appear.

The unwanted responses can be reduced or eliminated if the response of the east–west array is made negligible in these directions, since the cross response in any direction is proportional to the product of the responses of the east–west and north–south arrays. One way to do this would be to make the elements of the east–west array larger (in the north–south direction) than those of the other array. Since there are considerable advantages in making the elements of both arrays similar, a better solution is to break up the east–west array into two parallel arrays, spaced so that zero responses fall in the direction of the principal unwanted 'ripple' responses. Alternatively one may break up the east–west array into four parallel rows instead of two, and this may have economic advantages; the suppression of unwanted responses, moreover, is slightly superior to that of the two-row arrangement.

With this design, in which all elements are similar and have the same orientation, it is obviously possible to use the very economical frame and stretched-wire type of construction for the parabolic cylindrical reflectors[9]. A photograph of a model of such an antenna, with an off-aperture line-feed designed for accessibility and to reduce unwanted responses, is shown in Fig. 2. Fig. 3 shows the general arrangement of the cross-antenna in which four lines of parabolic reflectors, each 5,000 m. × 15 m. in aperture, are used for the east–west array, and 240 elements, each 85 m. × 15 m., make up the north–south array. The performance of this cross-antenna has been calculated, and it has been found that the discontinuous structure of the north–south array does not produce any significant deterioration in performance when compared with the theoretical performance of a uniform structure. On the other hand, the number of places at which current phases have to be changed is relatively small, and these can be individually monitored, so that one might hope to keep a more accurate phase control than would be possible with a uniform structure in which very large numbers of elements are involved.

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[3] Blum, E. J., *Ann. d'Astrophys.* (in the press).
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[6] Ryle, M., *Proc. Roy. Soc.*, A, **211**, 351 (1952).
[7] Messel, H., *Nature*, **188**, 528 (1960).
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ELECTRICAL PROPERTIES OF ORIENTATED LIPID ON A BIOLOGICAL MEMBRANE

AN ELECTROSTATIC DIFFUSION BARRIER AND ION PUMP

By Dr. J. W. L. BEAMENT

Agricultural Research Council Unit of Insect Physiology, Department of Zoology, University of Cambridge

THE layer of lipid over the cuticle of insects is said to contain an orientated monolayer of lipid molecules on a substrate of the tanned lipoprotein of the cuticle membrane[1–3]. It is this monolayer which is believed to provide both the extreme impermeability of the cuticle to water[2–4] and also the unusual wetting properties which the surface may exhibit[4–7]. It has been suggested that the polar ends of the lipid are strongly attracted to the tanned lipoprotein, so that the monolayer remains stable even when, as in aquatic insects, the non-polar ends of the lipid are in contact with liquid water for long periods[5]. Now it is known that certain types of monolayer film at an air/water interface may be associated with surface potentials[8]; but as such films become unstable if a further aqueous phase is brought into contact with their outer surface, investigation of their electrical properties is largely confined to the use of an air-electrode in the form of a radioactive source. If, however, the insect cuticle does have such a monolayer, it should produce an electric potential; since that monolayer is stable in the presence of aqueous phases on either side, then those properties should be readily measurable. Investigation of the electrical properties of the membrane therefore presents a means of testing the hypothesis that a monolayer occurs, for that hypothesis has been based entirely on the study of the relationship between the

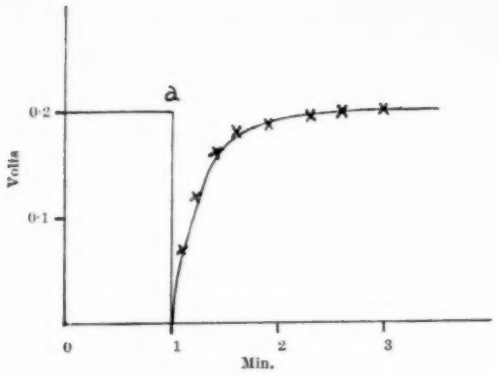

Fig. 1. Open-circuit voltage across a membrane preparation short-circuited for 5 sec. at *a*

membrane and water: either through measuring permeability or surface adhesion.

Electrostatic Potential across the Membrane

A membrane may be prepared from the integument of the cockroach *Periplaneta* by removing the cellular epidermis. When this membrane is arranged so that both sides are in contact with 1 per cent solutions of sodium chloride, there is a potential difference of the order of 200 mV. across the membrane, the morphological outside being positive with respect to the inside. Ten such membranes have given values between 170 and 235 mV., measured by a valve voltmeter of input impedance greater than 10^9 ohms. This potential depends on the grease (lipid) layer of the cuticle being intact, and can be destroyed by lipid solvents and by detergents. A potential difference of the same value is present across a cuticle membrane from which excess superficial grease has been removed by washing in the surface of clean water[2,5], that is, across a membrane believed to have only the orientated monolayer of grease on its surface. A similar electrical phenomenon has now been obtained with an artificial membrane coated on one side with grease from a cockroach (see below).

Using either a membrane with a natural thickness of grease (0·25μ) or with a monolayer, and with 1 per cent solutions of sodium chloride on either side, a small current will flow through a resistance connected between electrodes which dip into the solutions. If an ammeter of low resistance is used, a current of about 5 μamp. flows momentarily, and the potential across the electrodes falls very rapidly to an immeasurably low level. However, if connexion between the electrodes is then broken (except for the electrometer measuring the inter-electrode voltage) the original potential difference of 200 mV. reappears in the course of a few minutes. This process of recovery is exponential in form (Fig. 1), and by some 5–10 min. after 'shorting' the full potential difference has apparently been regained. The process of 'shorting' the electrodes may be repeated indefinitely—on each occasion the open-circuit potential of 200 mV. is re-established: one preparation has continued to behave in this fashion for 110 days with several hundred such treatments. But it is essential to maintain fresh solutions on both sides for recovery to be complete after short-circuiting in this fashion. If a high resistance is connected between the electrodes, then the potential difference across it falls rapidly to an intermediate value (for example, 50 mV. across a 22-M.ohm resistance in a typical experiment), and provided solutions of 1 per cent sodium chloride are maintained on both sides of the membrane, current continues to flow through the resistance. If the solutions on each side of the membrane are not maintained by perfusion, and the electrodes are connected through a resistance, the potential difference falls slowly. If the two electrodes are directly connected, and the preparation is left for several hours without perfusion, when the circuit is then opened, a lower potential difference than 200 mV. appears across the electrodes in the following few minutes.

Movement of Ions

In a typical experiment, which started with two identical solutions of 1 per cent sodium chloride on either side of a membrane initially developing 205 mV. potential difference, the electrodes were short-circuited for 24 hr., and the circuit then opened; the potential difference was 90 mV. It was found that there was a difference of 2·1 *p*H units between the two sides—the solution against the morphological inside of the membrane having become more acid. A difference of 2·1 *p*H units between two solutions corresponds to 120 mV. This, added to the 90 mV. remaining across the electrodes, gives 210 mV., which is, within the experimental error of the methods used, in agreement with the initial open-circuit voltage of 205 mV. across the membrane at the start of the experiment. If the two sides of the membrane are then perfused with fresh 1 per cent sodium chloride solutions, the original potential difference of 205 mV. may be obtained. It is clear from this and other similar experiments that ions are transported across the membrane to produce two different solutions when electric current flows through the membrane, that is to say, the device will transport ions against a concentration gradient. Stable equilibrium, in the sense that no potential difference may be measured between the two sides of the membrane, is reached when the concentration potential between the solutions on either side counterbalances the electrostatic potential on the membrane; but this is reached only after several days.

Origin of the Electrostatic Potential in the Membrane

Using a membrane with a thick layer (0·25μ) of grease on it, and 1 per cent solutions of sodium chloride on both sides, a voltage may be applied across the preparation by connecting a suitable generator, such as a battery. If the membrane is momentarily subjected to an externally applied potential difference of 6 V. of the same polarity as the 200 mV. naturally found on the membrane, an open-circuit potential difference of about 4 V. can immediately afterwards be measured. These excess voltages are here called 'over-charge'. If the applied voltage be of opposite polarity to the natural potential difference of the membrane, then the open-circuit potential difference immediately after treatment is found to be reversed (reverse-charge). Intermediate values of over-charge and of reverse-charge potentials can be produced by using smaller polarizing

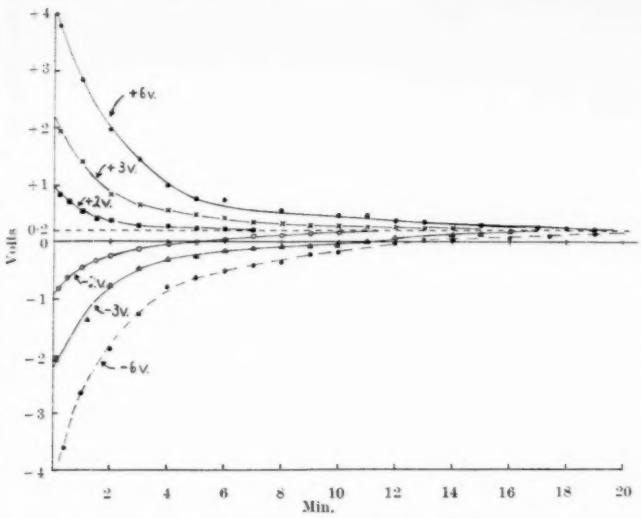

Fig. 2. Decay of over-charge voltages, and of reverse-charge voltages across a thickly greased membrane under open-circuit conditions. Labelling figures on each curve show the potential difference and polarity of the generator initially applied across the membrane to create the over-charge or reverse-charge. The untreated preparation has 200 mV. across it

voltages (Fig. 2), but potential differences in excess of 6 V. applied to the preparation will not raise the open-circuit voltage above 4 V. Both over-charge and reverse-charge potentials decay under open-circuit conditions over a period of about 20 min. to give the normal potential difference of 200 mV. (Fig. 2). ('Open-circuit' here means either with the valve voltmeter continuously attached to the electrodes, or momentarily attached to obtain potential difference values.) If, during the decay process, the electrodes are momentarily connected by a low resistance, the potential difference between electrodes falls to a very low level just as with a normal preparation (Fig. 1). And like a normal preparation, over the following minutes, the over-charged, or reverse-charged, preparation will produce a potential difference between electrodes when returned to open-circuit conditions (Fig. 3). However, the potential difference which reappears is that which the preparation would have reached by decay, if it had not been short-circuited. In other words, the process of recovery of the normal potential difference from conditions of over-charge or of reverse-charge is independent of the load placed across the membrane.

However, when an external voltage source is applied across a water-washed membrane (which has only the basal monolayer on it), no such temporary over-charged or reverse-charged states can be produced. The excess potential difference created on the thickly greased membrane must therefore be associated with the grease above the monolayer. When an artificial membrane is used, covered on one side with a thick layer of grease, or with a monolayer only, little if any potential difference can at first be measured when the membrane is mounted and perfused with 1 per cent solutions of sodium chloride on both sides. A small polarizing voltage (0·5–1·0 V.) applied across the membrane will suffice to produce a standing potential difference of about 200 mV. from this preparation, and then it will behave almost identically to a membrane taken from a cockroach. If the grease is thick, temporary overcharge phenomena can similarly be obtained. However, if a polarizing voltage of reverse polarity is applied to an artificial membrane which has only one monolayer on its surface, then a relatively stable potential difference of about 200 mV. occurs across the preparation, with reverse polarity, that is, with the greased side of the membrane negative to the ungreased side. The best artificial membranes so far produced do, however, lose their open-circuit potentials in the course of a few days, despite maintaining fresh 1 per cent solutions on either side. They can, however, be repolarized by an externally applied voltage at any time.

It is useful, in explaining these phenomena, to make reference to the phenomenon of the 'electret'[9] as well as to the surface potentials of monolayers at air/water interfaces. An electret is a material having

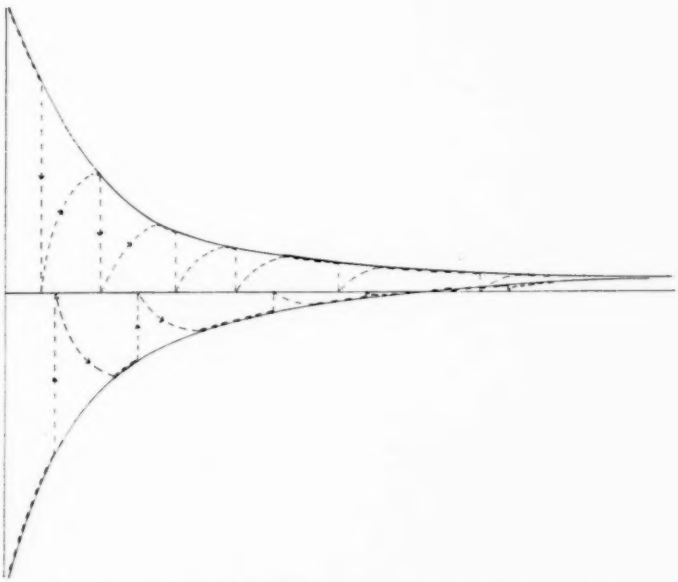

Fig. 3. Semi-schematic. The dashed line shows the form of recovery of the preparation of Fig. 2, over-charged or reverse-charged by 6 V., when repeatedly short-circuited momentarily during its decay. The natural decay is shown by the unbroken curves

a permanent electrostatic state, which is analogous to the permanent magnetic moment of a magnet. It has been known for many years[10–12] that a permanent electrostatic moment can be produced in a block of a suitable wax by melting it and allowing it to solidify in a powerful electric field; it is perhaps significant in the present context that beeswax is among the materials most suitable for making electrets, and it has indeed been suggested that it is the long-chain primary alcohols (such as are believed to comprise insect waxes[2,13]) which are essential components in electret-forming waxes. Although controversy still surrounds the explanation of some electretic phenomena in physical and theoretical terms[14–16], it would seem most satisfactory to assume that electron-displacement towards the polar group along the long chain of the alcohol molecule gives it its electrostatic moment. Crystallographic evidence has been advanced to support the idea that, in a hard wax electret, crystallites of wax are all aligned, whereas in normal wax they are randomly arranged, and that such alignment has been brought about in the molten wax under the influence of the applied electrical field, with the result that all the electrostatic moments are fixed, orientated, when solidification occurs[16]. A hard wax electret is virtually permanent. (Swann[17] suggests that slow thermal derangement of the domains in an electret will occur, producing degeneration, but that the half-life of the system is several years).

So far as the monolayer of grease on cockroach cuticle is concerned, the aligning force acting on the polar molecules is the high adhesion of the polar groups to the tanned lipoprotein substrate of the cuticle surface. Since this will also align the electrostatic moments of the molecules, an electret voltage would be expected to appear. It is interesting that a potential difference of 200 mV. across the probable thickness of the grease monolayer is the equivalent of an electrostatic gradient of 20,000 V./cm., which is precisely the highest electrostatic gradient quoted by several investigators of hard wax electrets[14,15,17]. Now according to the theories proposed from experiments on water permeability and surface properties[2–5,7], the bulk of the grease overlying the monolayer on a natural insect cuticle is not molecularly organized—on the cockroach it is a viscous fluid, and thermal agitation will ensure that the molecules are randomly arranged. Hence both the natural cuticle of the cockroach, with its thick grease, and also the cuticle washed by a water surface until only a monolayer remains, would be expected to produce the same electretic potential difference corresponding to one monolayer—which is found experimentally. If, on the other hand, an electric field is imposed on the thick grease, temporary alignment of all the grease molecules will be produced, and immediately after the application of such a polarizing field, the phenomena of over-charge or of reverse-charge, reported above, appear. The decay of these higher-energy states of induced molecular alignment is a direct consequence of the thermal derangement of the molecules in the thick grease (which may be from a normal or from an inverted orientation, depending on the polarity of the polarizing voltage which was applied); but the basal monolayer cannot be so deranged, and the preparation returns to a potential difference of 200 mV., regardless of whether it is short-circuited or not. The decay cannot, in fact, be influenced unless some further energy source is introduced into the system.

In a monolayer preparation, over-charge and reverse-charge potentials cannot be induced, because there is no thick grease to align. On the artificial membranes so far used, it is clear that the adhesion of the polar groups in the grease to the substrate is by no means as great as it is to insect cuticle. A combination of thermal agitation and electrical strain will thus prevent the formation of a good monolayer *ab initio*; but in an appropriately polarized electric field applied across such a membrane, a monolayer can be aligned—or indeed an inverted monolayer can apparently be stabilized. When this monolayer is inverted there are some indications that the surface adhesion of the monolayer to water is greatly increased, so that we have a direct confirmation of the theory of monolayer inversion and the production of hydrofuge surfaces already proposed[5]. However, it is seen from the experimental results cited here that, on an artificial membrane, the polar attractions to the substrate are not sufficient to maintain the monolayer against thermal and electrical strain, and it very slowly decays, whereas the structure on the more efficient substrate of cuticle is seemingly permanent. This adds to the conclusion already reached on different grounds[3,6] that considerable energy is needed in the construction by the insect of its monolayer.

Energies of the Ion Pump

It must be clearly understood that a monolayer, with its distribution of charge, does not represent a 'battery' or source of energy, any more than a hard wax electret does. In the case of the hard wax electret, current will flow temporarily through a wire connecting two plates applied to the two sides of the block of wax. If the plates are repeatedly removed and replaced, current may repeatedly be drawn: the energy for this electric power is put into the system by the process of applying and removing the plates. In the case of the cell, made up of a permanently charged membrane, with identical solutions on either side, current can only be obtained in the external load for so long as the two solutions are maintained. Consider the well-known phenomenon of the diffusion or concentration cell (Fig. 4*A*). A potential difference is generated between two solutions of different composition separated by a membrane which is electrically inert, but through which components of the two solutions may diffuse. The potential difference between the two solutions is determined by the difference between the composi-

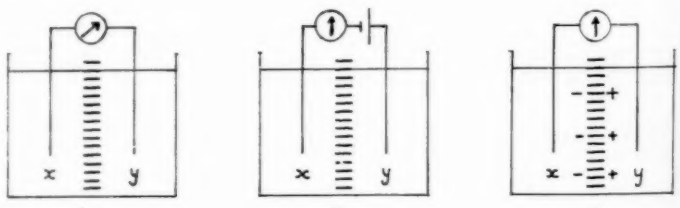

Fig. 4. See text

tions of the two solutions; it disappears when the solutions have become the same through diffusion, and the system has reached a state in which there is no thermodynamic difference across the membrane. However, if the changes in the two solutions, due to diffusion through the membrane, are countered by perfusing the two sides of the membrane with their respective original solutions, the potential difference across the membrane can be maintained indefinitely; energy is introduced into the system in removing the products of diffusion.

If a source of potential difference (not of energy) is inserted into the leads connecting the electrodes of such a cell (Fig. 4*B*) current does not flow in the external circuit when the two solutions are at compositions which are not identical, but which are such as to generate a potential difference equal and opposite to that of the introduced source of potential This represents the equilibrium state in which energy cannot be derived from the difference between the solutions. However, this situation is electrically identical to one in which such a source of potential is introduced at another point in the circuit—across the membrane (Fig. 4*C*). It follows that if identical solutions are placed on both sides of a membrane with an electrostatic potential across it, the composition of these solutions will tend to change until they establish an electric potential exactly compensating that on the membrane, and that this condition will represent thermodynamic equilibrium in the system. It further follows that, in the device described in the early part of this article, the source of energy for the continuous flow of current through an external load is provided by the perfusing solutions of 1 per cent sodium chloride, in displacing the concentration difference which the membrane is endeavouring to establish on either side of itself.

The biological consequences of this system cannot as yet be fully foreseen. An electrostatically charged monolayer of lipid is capable of maintaining two solutions of different composition, concentration, *p*H, etc., on either side of itself, without the expenditure of energy, that is, a thermodynamic device of great biological advantage; it must be remembered that this it can only do because of the enormous amount of potential energy stored in the monolayer, both in its orientation order and also in its bonding to the protein substrate. Maintenance is not, however, necessarily a static matter; if the solutions on either side change from such compositions as to offset the membrane potential, ions may be transferred to counter that change. The membrane may, therefore, be the integral part of a biological 'ion pump'; the energy for this pump is not derived from any 'carrier' used in the transport process, but is a direct consequence of the organization of the membrane itself. Biologically speaking, the energy for using the pump might be provided by the manipulation of the concentrations of materials on either side of the monolayer. It is appreciated that, according to current ideas, cell membranes contain a bimolecular leaflet of lipid, rather than a monolayer, but it would be necessary only for there to be a net electrostatic field from the algebraic sum of the moments of the monolayers comprising the leaflet, for the system outlined in this article to operate; of course, across a complex differentiated bimolecular layer, local variations in potential and polarity might be very considerable.

I am grateful to Drs. R. H. J. Brown, K. E. Machin and J. W. S. Pringle for discussions during the course of this work.

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VIRTUAL ABSENCE OF GLUTATHIONE INSTABILITY OF THE ERYTHROCYTES AMONG ARMENIANS IN IRAN

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AND
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THE glutathione (GSH) stability test of the erythrocytes developed by Beutler[1] and studies by the University of Chicago group[2] have resulted in the discovery of a genetic erythrocytic defect, namely, an absence or a deficiency of the enzyme glucose-6-phosphate dehydrogenase. This erythrocytic defect is found in some ethnic groups but not in others[3–6]. In general, American and North European Caucasians, Askhenazic Jews, American and Peruvian Highland Indians and Japanese do not have the defect. However, American Negroes, Sephardic Jews, Sardinians, East and West Africans, and some Asians, have the genetic defect to an extent such that tests for deficiency of this enzyme have become valuable anthropological tools. Recently we reported that approximately 8 per cent of Moslems in Iran are sensitive by the GSH stability test[7]. Several tribal groups studied thus far have also shown incidences of the

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defect similar to the Moslem group and even higher, for example, an incidence of 15 per cent in the Mamassani tribal group[8].

This report is concerned with an examination of 163 Armenians, 153 of whom reside in New Julfa near Isfahan. The subjects comprised 152 males and 11 females. Of this group, only one male was found to be sensitive by the GSH stability test of the erythrocytes, making this population the first thus far studied to be virtually negative for the defect in Iran. Unfortunately the one 'sensitive' person could not be traced for requestioning as to possible admixture with another ethnic group. Blood samples were taken from apparently healthy subjects only, but this does not exclude the possibility that this male could have had congenital non-spherocytic hæmolytic anæmia, which is another condition where erythrocytes from such persons also show the enzymic defect[8]. The virtual absence of the genetic defect in Armenians is of greater importance than the finding of the defect in this ethnic group.

According to Montague[9], Armenians are a composite of an Atlanto–Mediterran component and a greater Irano–Afghan element with some Nordic and Alpine elements. From studies of *ABO* blood groups published thus far[10], the Alpine component is very strong for there is an extremely high frequency of the *A* gene. This was confirmed in our *ABO* studies on this population (gene frequency : p, 0·3894 ; q, 0·1084; r, 0·5022, D/σ, + 0·3). The *cde* gene frequency was 0·3941, which is also European. These studies of blood groups of Armenians are distinctly different from those of Boué and Boué[11] on other populations in Iran and our own unpublished observations.

The Armenians once occupied a large part of the area which was the seat of the Hittite Empire (present-day Turkey) and they are believed to be descendants of these people. It is of interest to note that authorities are divided as to whether the Hittites, with their Indo-European language, came originally to Anatolia about 2,000 B.C. from the north-west from northern Europe or from the north-east across the Caucasus Mountains[12]. Be that as it may, there can be no doubt that there is a distinct northern European influence in Armenians from the present study and those of other workers. This northern European influence is strong, despite the fact that the Armenians throughout their long history and by virtue of being situated along the trade route between East and West have been in association with diverse ethnic groups which have and probably did have the gene for glucose-6-phosphate dehydrogenase deficieney, such as Phœnicians, Africans, Jews, Greeks and Iranians.

The Armenians in New Julfa were brought to Iran as Christian captives in 1604 and 1605 by Shah Abbas the Great from Julfa in what is now Soviet Armenia[13,14]. Rather than being treated as slaves, they were encouraged to create a prosperous trade centre between East and West in the area of Isfahan. The original number was about 25,000 ; the present population is about 6,000[14]. The decrease in size is due to movement of many to other parts of Iran and to other countries, mainly India. While religious intolerance has not been permitted in Iran for many centuries, the Armenians were not encouraged to intermarry with the surrounding population. According to Dr. Caro Minasian, an Armenian physician and scholar, Armenians who have intermarried live outside of the area of New Julfa. This fact is supported by our finding of a virtual absence of the erythrocytic defect and by the blood group data. This population is thus an excellent example of what has been termed 'religious isolates' by Montague[9]. Since the original group brought to Iran was fairly large, it is unlikely that the genetic defect was present in the population and then lost during the last 35[3] years as a result of 'genetic drift'.

Of further interest is the fact that both the area in which the Armenians now live and their original homeland have been, until recent years, regions of high endemicity so far as falciparum malaria is concerned[15]. Several reports have noted that the gene for glucose-6-phosphate dehydrogenase deficiency coincides with the incidence of falciparum malaria in many parts of the world and have suggested that this harmful gene might possibly be maintained as a result of balanced polymorphism with respect to this disease[6,16]. The Armenians in the Isfahan region represent one group which has resided in a malarious area for centuries without the appearance of this protective effect.

During the course of the survey, the subjects and others in the area were questioned about the ingestion of fava (broad) beans, which are agents having an adverse effect on carriers of the genetic defect[8]. Although practically all of them ate fava beans regularly in the spring of the year, neither the subjects nor the physicians in the New Julfa area were aware of any difficulty as a result of eating this food. This is unlike our experience with the Moslem and Jewish groups in Iran. Most of these people are familiar with the possible harmful effect of the *Vicia faba* bean.

It appears to be very important that other workers who have access to Armenians should make studies comparable with ours. The findings of either a high incidence of the genetic defect or a virtual absence such as we have found in Armenians in New Julfa would be of great interest to anthropologists and geneticists.

We wish to express our appreciation to Dr. Caro Minasian for making this survey possible and for his clarification of many points regarding the history of the Armenians. We thank Messrs. Galoost Galestian, Wayburn D. Davies and Parviz Haghighee for assistance on the survey, Misses Annerose Oemisch and Iran Haffarian for technical assistance in the laboratory and Dr. Garegin Saroukhanian for his translation of several Russian sources on malaria in Soviet Armenia.

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OBITUARIES

Prof. H. W. Turnbull, F.R.S.

By the death of Prof. Herbert Westren Turnbull at Grasmere on May 4, Britain has lost a distinguished mathematician of the older school, while many mathematicians in England and Scotland have lost a personal friend.

Herbert Turnbull, a son of W. Peveril Turnbull, H.M. inspector of schools, was born in Wolverhampton in 1885. From Sheffield Grammar School he went as a scholar to Trinity College, Cambridge, being Second Wrangler in 1907, first in the first division of Mathematical Tripos II in 1908, and Smith's Prizeman in 1909. After a varied experience of lecturing at Cambridge, Liverpool and Hong Kong, he seemed for a few years to be likely to follow his father's career, teaching as he did at Repton for three years and becoming an H.M. inspector of schools. This pattern was quite altered in 1921 by his appointment to the chair of mathematics in the United College, University of St. Andrews.

The result was to associate his name permanently, and especially to Scottish mathematicians, with St. Andrews. He wrote there the great majority of his sixty or more published papers, and founded a school of algebra and geometry of the older type which, though younger mathematicians have moved to the more modern abstract disciplines, had value in its day. Turnbull was inspired immediately by J. H. Grace and Alfred Young, his associates and seniors by slightly more than a decade at Cambridge, and through them by the Continental masters Clebsch, Gordan and Capelli, with Cayley and Sylvester beyond again. The realm most congenial to him was that of the invariants and covariants of algebraic forms, as investigated by the Clebsch–Aronhold symbolic calculus, of which he became the last and in some ways perhaps the greatest master; but he was constantly aware of the applications to projective geometry. Also, when in 1927 Alfred Young began his new attack on invariants with the Frobenius–Schur group algebra of the symmetric group, Turnbull was fully alive to the new range thus opened, and some of his work consists of a reconciliation of the symbolic with the group-algebraic approach. His name will also be associated with memoirs and text-books on matrices and determinants.

An interest in the history of mathematics first found utterance in a small book, *The Great Mathematicians* (1929); but the discovery of hitherto unknown documents relating to the work of the Scottish mathematician, James Gregory (1638–75), produced his first historical work on a larger scale, the *James Gregory Tercentenary Memorial Volume*. Felicitously, this was ready in time for the James Gregory tercentenary celebrations which, somewhat resembling the Napier tercentenary celebrations of 1914, took the form of an international congress of the Edinburgh Mathematical Society—the University of St. Andrews and the Royal Society of Edinburgh being sponsors. This occasion, too soon overshadowed by rumours of war, will be remembered by many as the special one in which Turnbull, in the prime of life and ably seconded by his wife, showed himself as the host of hosts and moreover as an excellent musician, for he was a pianist of almost professional standard. To Scottish mathematicians these qualities were well known, in the annual June meeting of the Edinburgh Mathematical Society at St. Andrews and its four yearly colloquia held there.

Retiring in 1950, Turnbull as emeritus professor devoted himself to his second and major historical work, the editing of the correspondence of Isaac Newton. He lived to see the first volume, handsome and impressive, appear in 1959, and to know that the second was well under way.

His character was founded on the simplest faith, absolute sincerity and the desire to serve. His many friends, in their sympathy extended to his wife and their son Derwent, will remember the man first, and then the mathematician. A. C. AITKEN

Prof. C. G. Jung

CARL GUSTAV JUNG, who died on June 6, was born on July 26, 1875, at Kesswyl, in Switzerland. His father was a clergyman and he was brought up in his parsonage in the country. He attended school at Basle and was educated in medicine in that city, where he graduated.

In 1900 he became assistant in the State Mental Hospital and in the psychiatric clinic of the University of Zurich, later becoming senior staff physician there until 1909.

In 1902 he spent six months in Paris attending P. Janet's lectures and worked in Zurich with E. Bleuler. He first met Freud in 1906.

When Freud put forward his ideas and discoveries he had been greeted with scorn and derision until Bleuler made it known that his views were regarded sympathetically by the Zurich school. The interest of the Swiss certainly helped to direct the scientific world towards psychoanalysis, and Freud acknowledged his gratitude, stating, "The Zurich doctors made, as it were, the nucleus of the little band which was fighting for the recognition of psychoanalysis. Only in Zurich was there a possible opportunity to learn the new art and to apply it in practice".

Freud at this time wished to take a less prominent place in the Psychoanalytical Society and diplomatically transferred his presidency to Jung.

Jung must have been an impressive young man in those days, and Wittels described him in his well-known depiction as "Tall and upright of figure, like a young Siegfried". When I first saw him some thirty years ago he was middle-aged, but still well built, with a rather burly figure, a jovial red face, short clipped hair, and gold-rimmed glasses. He was a good lecturer and able to impress an audience with his profundity. With those who sought his views he had a kindly, serious manner and he was certainly 'a good mixer'.

The first important investigation of note which he made was his careful study of association. His research followed on the experimental work started by Galton in 1879 and Wundt a year later. This was 'controlled association' or the timing of words associ-

ated with those already provided. This led to the development of the conception of 'the complex' or group of ideas, emotionally charged, capable of influencing conduct and thought. Following on his work on association Jung did some valuable research on schizophrenia, published under the title of *The Psychology of Dementia Precox* (1909).

He remained president of the International Psychological Society until 1913, when he published his book on *The Psychology of the Unconscious*, which was a study of mythology. This showed such a deviation from psychoanalysis that he resigned. The Zurich and Vienna schools then drifted apart.

After this Jung advanced his well-known theory of psychological types. This was a development of philosophical views known from the earliest times and used by William James, who thought men were divisible into tough and tender, by Schiller who thought them idealists and realists and so on. However, the two main types Jung described were extroversion and introversion, but complicated by the addition of sub-classes of thinking and feeling, so that there were eight in all. Extroversion and introversion have become household words.

Jung moved further and further from clinical psychiatry and more and more towards an interest in mythology. His later works were, *The Secret of the Golden Flower* (1930), *Modern Man in Search of His Soul* (1933), *Psychology of Religion* (1939), and *The Integration of the Personality* (1940), etc.

Although Jung's investigation of myths is regarded with suspicion by many psychiatrists, since it is impossible to prove the correctness of an interpretation, and the application of myths to the understanding of clinical illness is of uncertain value, most accept that Jung's earlier work on association, complexes, schizophrenia and psychological types is of immense value.

He was a tireless writer and lecturer. He visited most countries at the invitation of learned societies and studied myths all over the world. The last of the triumvirate formed by him with Freud and Adler, his death represents the passing of a great epoch of psychiatry.

CLIFFORD ALLEN

NEWS and VIEWS

Electrical Engineering at Glasgow : Prof. J. Lamb

DR. JOHN LAMB has been appointed to the James Watt chair of electrical engineering in the University of Glasgow, in succession to the late Prof. B. Hague. For the past five years Dr. Lamb has been a reader in electrical engineering in the Imperial College of Science and Technology, London. He is well known for his many contributions to knowledge in the fields of ultrasonics and the physics of materials. Dr. Lamb is thirty-nine years of age. He graduated in electrical engineering in the University of Manchester in 1943, and was awarded the degree of Ph.D. in 1946 and the degree of D.Sc. in 1957. In 1947 he joined the staff of the Imperial College. For the past three years, in addition to his readership, he has held the appointment of assistant director in his Department, involving administrative responsibilities that will no doubt have given him experience that will be of value in taking charge of a department. It may be expected that the appointment of Dr. Lamb will ensure that electrical engineering in Glasgow will be developed on modern lines and with a keen appreciation of the part that advances in basic physics must play in the future of electrical engineering.

Beilby Medal and Prize, 1961

THE administrators of the Sir George Beilby Memorial Fund, representing the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals, have decided to make awards from the Fund in 1961—each consisting of the newly instituted gold medal with a prize of 100 guineas—to Dr. Constantin Edeleanu and Prof. Jack Nutting.

Dr. C. Edeleanu

DR. C. EDELEANU was born in Rumania, and went to Britain in 1937. He was a pupil at the Morrison Academy, Crieff, and then went up to Cambridge in 1941. In 1946 he joined Dr. U. R. Evans, working on the stress corrosion of light alloys. In the course of that work he became interested in pitting reactions and developed the autocatalytic mechanism for pitting first proposed by Dr. Hoar. A somewhat surprising conclusion of the work was that the true rate of corrosion during the pitting of aluminium in a given solution was a constant. Direct experimental proof of this was, however, only obtained some ten years later. In 1950 he joined the Brown Firth Research Laboratories, where he was in charge of a section dealing with long-term alloy development. Stress corrosion, especially in high-pressure water systems and nuclear-reactor cooling systems, was of particular interest, and the work demonstrated that failures in such systems were, as a rule, associated with concentration effects arising either during heat transfer or in heating and cooling cycles. In 1956 he joined the Tube Investments Research Laboratory at Hinxton, to form a corrosion group. At first the work consisted mainly of studying the factors that control corrosion in some fused-salt systems of interest to the nuclear-engineering industry. One of the main results was to show that such systems could be treated in a somewhat analogous way to aqueous systems but that there were important differences. More recently, Dr. Edeleanu has returned to stress corrosion and, in conjunction with Dr. A. J. Forty, has attempted to interpret this type of failure in a more physical manner. This led naturally to the question of the part played by dislocations and other structural features in corrosion. With G. A. Bassett a start was made in this field, techniques such as transmission electron microscopy having played a useful part.

Prof. J. Nutting

PROF. J. NUTTING was educated at Mirfield Grammar School, Yorkshire, and the University of Leeds. He was then appointed research assistant to the late Prof. Preece at Leeds and was awarded the degree of Ph.D. in 1948 for a dissertation on the overheating and burning of steel. Afterwards he was employed by the British Iron and Steel Research Association and was seconded to the Cavendish Laboratory, Cambridge, where he worked with Dr. V. E. Cosslett and Prof. E. Orowan, developing tech-

niques for the examination of metals with the electron microscope. In 1949 he was appointed demonstrator and in 1954 lecturer in the Department of Metallurgy, University of Cambridge—an appointment he held until 1960, when he returned to the University of Leeds as professor of metallurgy in the Houldsworth School of Applied Science. During his work at the Cavendish Laboratory he became convinced that the electron microscope could be more widely applied to problems in metallography. On transferring to the Metallography Department in Cambridge he had the opportunity of testing his convictions, and together with a group of research students a wide variety of metallographic problems was investigated.

Paul Instrument Fund Committee Grants

Awards of grants by the Paul Instrument Fund Committee have been made as follows: £1,550 to Dr. E. T. Hall, director of the Research Laboratory for Archæology and the History of Art, Oxford, for the design and manufacture of a recording instrument combining a drum recorder and servo transmitters with a spectrometer to give a direct reading of wavelength; £3,900 to Dr. P. B. Hirsch, lecturer in physics, University of Cambridge, for the construction of an electron optical instrument which will measure the energy distribution of the electron beam passing through small areas (diameter less than 1μ) in the crystal specimen which can be observed and selected on the electron microscope image; £3,000, in supplement of a previous grant, to Dr. H. Motz, Donald Pollock reader in engineering sciences, University of Oxford, for the construction of a linear accelerator working at 1·6 cm. (*J*-band); £2,284 to Mr. B. M. Turner, who is working in the Department of Zoology and Comparative Anatomy, Oxford, for the further development of micro-scale chromatography and electrophoresis techniques.

The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

U.S. Scientists in Universities and Colleges : 1958

A preliminary report on scientists and engineers engaged in research and development in colleges and universities in the United States for 1958, issued by the National Science Foundation (*Reviews of Data on Research and Development*, No. 27; April 1961), gives the number of those engaged as 69,919 in 377 colleges and universities, or 44 per cent of all scientists and engineers employed at colleges and universities in 1958, and the expenditure as 736 million dollars, compared with 410 million dollars in 1954. Of these, 47 per cent were employed in the biological sciences, 26 per cent in the physical sciences, 17 per cent in engineering sciences and 10 per cent in the social sciences. Operating expenditure for separating budget research and development at the universities and colleges accounted for 45 per cent of the total expenditure on research and development, agricultural experiment stations for 16 per cent, and Federal Contract Research Centres for 39 per cent. Sixteen institutions employing 1,000 or more employed 31,260 of this total of scientists and engineers, and twenty institutions with 500–999 another 14,026, while 206 institutions employed fewer than 25 scientists and engineers. While the total faculty engaged in research and development only rose by 3 per cent between 1954 and 1958, the number of faculty engaged full-time in research and development increased by 49 per cent.

Production of Gas from Coal in Britain

In a written answer in the House of Commons on June 23, the Minister of Power, Mr. R. Wood, said that he had accepted the advice of the Wilson Committee that town gas offered the greatest scope for expansion in making gas, oil and chemicals from coal, but that new plants would be required to make gas more cheaply. He had therefore decided, in agreement with the Gas Council and the National Coal Board, to accept the recommendations that the development of high-pressure slagging gasifiers should be pursued; that the Gas Council should be responsible for further work on the standard Lurgi gasifier using British coals, on the Otto–Rummel gasifier and on any further gasifiers suitable for town gas, as well as for development on slagging gasifiers; and that the National Coal Board should be responsible for work on producer gas plants. He also accepted the Committee's view that the prospects of making oil from coal economically in Britain are so remote that no further technological work on the development of this process should be undertaken at present. The Department of Scientific and Industrial Research had accordingly terminated its development work on this process, but was continuing basic investigations on catalysis and keeping in touch with developments in making oil from coal. The Minister said that his Department, which had been carrying out research and development on the slagging gasification of coal as a raw material for oil synthesis, would continue this work until March 31, 1962, when the Gas Council would be responsible for all work in this field. The Gas Council and the National Coal Board were making a joint economic study of the possibilities of the Lurgi process which might later be extended to include slagging gasification. This study would define the scope for further developments of the process and provide a basis for further co-operation between the Gas Council and the Board in the total gasification of coal.

Research on West Indian Volcanoes

Geological work is in progress on volcanoes in the West Indies, by the Department of Geology and Mineralogy, University of Oxford, in co-operation with the Seismic Research Unit of the University College of the West Indies. In view of the large amount of geophysical data available on this region, it is desirable that a detailed petrological study of the volcanic rocks of the British Leeward and Windward Islands be undertaken. A succession of Oxford graduate students will work on specific problems during the next five years, under the supervision of Prof. L. R. Wager and Dr. G. M. Brown (University of Oxford) and with assistance in the field by Dr. G. R. Robson (Seismic Research Unit) and Dr. P. H. A. Martin-Kaye (Government Geologist, Windward Islands). Their work will include mapping of the structure of the volcanoes and the recognition and classification of the various phases of recent volcanic activity. Petrological and mineralogical

work on the rocks and minerals will, in conjunction with the field work, lead to a better understanding of the origin of the rocks and of some of the causes underlying the development of volcanic episodes in the region.

Work has begun with studies by Prof. Wager on the petrology and mineralogy of plutonic blocks ejected by the Soufriere Volcano, St. Vincent (1959 visit), and by P. E. Baker on the strato-volcano, Mt. Misery, on St. Kitts (1961 visit). Other students will begin work shortly on St. Lucia and Dominica, which are characterized by having centres of activity concentrated in almost circular caldera-type depressions, in which tholoids and ash deposits predominate and in which fumarole activity and earth tremors have persisted in recent years.

Education by Television

As a tool for instruction, television is accepted and well established in the United States. Television made its first appearance in education as long ago as 1932 at Iowa State University, but it was not until 1953 that it was first used for normal instruction. By 1960, half a million school-children and 100,000 college students were receiving regular instruction by television. This situation is in marked contrast with that prevailing in Great Britain. In a pamphlet entitled *Educational Television: a Survey and a Plan*, which has been published privately, John Wellens shows that in Britain educational television is practically synonymous with the schools television service run by both the B.B.C. and the independent network (Pp. 44+8 plates. Guilsborough, near Northampton: John Wellens (ITT), Ltd., The Sun House, 1961. 5*s*.). The only exception consists of a handful of colleges and universities which have their own closed-circuit installations for giving instruction in special departments, such as medicine, dentistry and engineering. These installations are few and have only recently been introduced; in many cases they have not yet developed beyond the pilot stage. In some cases the installations have been put in, below economic cost, to promote the acceptance of television as a teaching tool. Two Middlesex schools have been linked by micro-wave equipment for the teaching of chemistry, but, none the less, Britain is now at the stage reached by the Americans approximately ten years ago. Within the past year British educationists have become aware of the activity in this field in the United States and, in his booklet, Wellens describes the present position in the United States and makes a practical evaluation for Britain.

Research Defence Society

The Research Defence Society has published another of its pamphlets to make known the facts about experimental research involving the use of animals, and the conditions and regulations under which animal experiments are conducted in the United Kingdom. *Animal Experiments in the Control of Animal Diseases* is set out on the lines of previous pamphlets, that is, by posing a series of questions which the general public could rightly ask and by providing answers which are as objective and free from bias as humanly possible (Pp. 6. London: Research Defence Society, 1961. 6*d*.). In this new pamphlet the questions posed include: "Why are the treatment and prevention of animal disease important enough to warrant animal experiments? Why is animal experiment necessary to help in combating animal disease? Can you give examples of new agents for control of animal disease which would not have been developed without animal experiment? Can you explain the paradox that animal experiments involving suffering are used to prevent suffering in other animals? Why are animal tests necessary in the development of new drugs? How do animal experiments help in the control of animal disease? Why are animal experiments necessary in the safety testing of vaccines for use in animals?"

The Museums Journal

Earlier this year it was decided to discontinue the monthly *Museums Journal* and publish instead a monthly *Bulletin* and a quarterly *Journal*. The first issue of the latter publication (No. 1; June 1961) was awaited with interest. The decision of the Council is fully justified, and curators are certainly not disappointed, for the new format even exceeds expectations. Mr. R. L. S. Bruce-Mitford describes the Book of Kells exhibition, stressing the technical presentation; Mr. Ronald Alley, in a comprehensive analysis, proves the almost total failure of British art galleries to acquire modern pictures and sculptures of foreign schools; and Mr. Stanley Rubenstein deals with the complicated problems of copyright. On the purely technical side, Mr. R. M. Organ gives a new treatment for 'bronze disease', and Mr. A. M. Tynan describes the uses of 'Styrocell'. Mr. Hugh Wakefield details the practical nature of the purchase grants made by the Victoria and Albert Museum. The issue also includes the annual report of the Museums Association for 1960–61, and a well-balanced *Journal* concludes with notes on the contributors and research work in progress.

The Victorian American

In 1960 the family of the late Harry T. Peters presented the 'American on Stone' collection of lithographs to the Smithsonian Institution (Publication 4466: *The Victorian American*. By Antony N. B. Garvan and Peter C. Welsh. Lithographs from the Harry T. Peters 'American on Stone' Collection. Pp. 30. Washington, D.C.: Smithsonian Institution). The collection touches on every phase of American life in the nineteenth century. Part of this interesting material is now on tour as a travelling exhibition, and the 100 lithographs listed in a well-prepared catalogue include those illustrating technical progress, suburban novelty, rural nostalgia, the family and the genteel female, sports, fashion, the sea, politics, warfare and humour.

Medical Research in East Africa

A new treatment for tuberculosis and investigations into the causes of eye diseases in Africans are among the many items referred to in the report of the East African Council for Medical Research for 1959–60 (Pp. ii+15. Nairobi: Government Printer, 1960. 2*s*.). In tuberculosis, the important finding has been that thiacetazone (thiosemicarbazone), TB1, is as efficacious in combination with isonicotinic acid hydrazide (INAH) in therapy and in preventing drug resistance as is *para*-aminosalicylic acid (PAS). TB1 is cheap and palatable; PAS is expensive and patients dislike it. They are apt, therefore, to take INAH alone and thus acquire resistance to infections. A high (about 10 per cent) and increasing incidence of initial drug-resistance has been revealed in new and presumably untreated cases. The investigations now

in progress are to determine the optimum dosage. Surveys have been carried out to determine the most common eye conditions in different African communities. A higher incidence of non-leprous eye disease was found in lepers in the Central Province than in Lake Province, and this is probably due to trachoma and pterygium being common in the former area. In the foothills of the Ruwenzori Mountains of West Uganda, onchocerciasis is common; it was found that this filarial infection was the most important cause of blindness. Some investigations into the ability of the African to accommodate were made. Although during early life accommodation is poor, it does not deteriorate so rapidly in late middle-life as it does in Europeans. Colour vision was also tested, and it was confirmed that the incidence of red-green and other forms of colour-blindness is lower in Africans than in Europeans.

Diabetes Mellitus in Africans in Rhodesia

A SURVEY carried out recently on Salisbury Africans by Dr. M. Gelfand and W. R. Carr indicates that diabetes is less prevalent than in most other parts of the world, particularly in comparison with areas where more processed and highly refined foods are purchased (*Central African Journal of Medicine*, **7**, No. 2; February 1961). The diets with traditionally high carbohydrate, low fat and low animal protein contents are probably the main factor involved in keeping the disease at a low level. There are increasingly large numbers of Africans whose diet is changing tremendously during short periods of time towards higher fat, refined sugar and animal protein consumption, but, as yet, they are insufficient in numbers to indicate whether this will have an effect on the incidence of diabetes and, *inter alia*, coronary thrombosis. The socio-economic background shows that most of the diabetic patients come from groups who work in the European economy, and whose diet has been altered to include more foods containing, in particular, refined sugar and fats. The clinical types encountered in Rhodesia correspond very closely with the European pattern. No variants such as *J* or *K* types are found. The African diabetics may require more insulin than the European for stabilization, but this may be due to lack of understanding of the need to follow a strict dietary routine. The level of blood-sugar was found to be higher than in a European group and this may be the reason for the difference. Although the insulin requirements of the African appeared to be high, hypoglycæmia coma developed if the requirements were exceeded. The symptoms of diabetes appear to be the same for the African and European, the main features being thirst, polyuria, weakness, loss in weight and pruritus vulvæ; but there were certain differences, such as the infrequency of pulmonary tuberculosis, gangrene and intercapillary glomerulosclerosis.

Ecological Aspects of Irrigation in Ancient Mesopotamia

A PAPER by Hans Helbaek, "Ecological Aspects of Irrigation in Ancient Mesopotamia" (*Iraq*, **22**, 186; Spring and Autumn, 1960. Published by the British School of Archæology in Iraq), develops the argument that man first harvested his grains (barley and linseed, in particular) where the wild plants grew on hills in the Near East, and on soils fed by rain. Here he supposes the genetic constitution of the crop plants remained fairly constant. But some 7,000 years ago man sowed and grew his wild barley in the plain of Mesopotamia in rich land naturally irrigated by flooding (and later artificially irrigated by canals). Here presently the wild 2-row barley gave rise to a 6-row barley by a moderate genetic change causing the two lateral abortive spikelets to become fertile. Helbaek claims to have produced such a barley as a mutant from a 2-row wild barley by X-ray irradiation. Such 6-row barley was welcomed by early man, and spread to Egypt and elsewhere. Meanwhile, continued irrigation in Mesopotamia led to salt accumulation in the soil and the disappearance of crops, first of the sensitive Emmer wheat, then linseed, and finally of barley, and with that of man. Helbaek gives evidence also that while early man's *Linum* seeds (whether flax or linseed) remained constant in size for some thousand years by areas fed by rainfall, the size of seed steadily increased in areas with irrigation. Readers interested in the botanical history of crop plants and of the early growth of civilization made possible by crops should read and assess this short paper themselves.

Taxonomy of the Earthworm in India

EARTHWORMS, apparently sent simultaneously to the British Museum (Natural History) and to the American Museum of Natural History for identification, have directed attention once more to the deplorable state of the taxonomy in a genus that may be dominant in a considerable portion of the Indian Peninsula. Each of the species left in that genus by a revision by G. E. Gates in 1939, based on an over-all somatic rather than genital anatomy, has remained indefinable hitherto. Material now studied by Gates, at the University of Maine, has enabled recognition not only of two further characters for generic definition but also has permitted, for the first time, fairly satisfactory characterization of a species based on data such as variation in sexual individuals secured in lots of some size from several localities (*Ann. Mag. Nat. Hist.*, **3**, No. 35; November 1960). Previously unpublished information included in his account was obtained from 90 specimens collected for him at three localities in the Jubbulpore region during the Second World War.

Geology of the Country around Monmouth and Chepstow

THE Geological Survey of Great Britain has now published a composite Memoir covering the geology of the country around Monmouth and Chepstow (Department of Scientific and Industrial Research Memoirs of the Geological Survey of Great Britain—England and Wales. By Dr. F. B. A. Welch and Dr. F. M. Trotter. With contributions by T. R. M. Lawrie, J. D. D. Smith and R. W. Elliot. Pp. viii+164+2 plates. London: H.M.S.O., 1961. 22*s*. 6*d*. net). Previously, this area was dealt with in separate Memoirs and Survey sheets, but the present Memoir is an explanation of New Series Geological Sheets 233 (Monmouth) and 250 (Chepstow)—an area occupying a total of 432 square miles, of which some 46 square miles are covered by the Severn Estuary; a little more than half lies within Monmouthshire, part in Herefordshire and the remainder in Gloucestershire. The Memoir details the geological sequence and history of the area, which extends from the Ludlow Series of the Silurian rocks through Old Red Sandstone, Carboniferous, Triassic, Jurassic to recent superficial formations. A general account of the

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geological structure of the region is included, also an analysis of those earth movements which have affected the area. Each geological epoch represented is treated individually, and bibliographies of the literature are provided. A short chapter deals with the igneous rocks of the area, and another concerns itself with the economic deposits. Two appendixes provide records of recent important bore-holes, and also a list of Geological Survey photographs of the area.

The Art of Interviewing

The British Association for Commercial and Industrial Education has produced a pamphlet which should find readers well outside the range of those for whom it was intended. Written by John S. Gough, of I.C.I., Ltd., the pamphlet *Interviewing in Twenty-six Steps* describes the approach to an interview designed to select a candidate for a job, the method of conducting it, the dangers and diversions to be avoided and the way the interview should be used in summing-up the candidate. The pamphlet is forthright, practical and has clearly been written by one who has tried out his twenty-six steps on many occasions before summarizing them in this form. Gough makes it clear that no skilled interviewer can guarantee success in selection; the more he practises, the less mistakes he should make. This pamphlet will help him to reduce his number of failures. Copies may be obtained from the British Association for Commercial and Industrial Education, 26*a* Buckingham Palace Road, London, S.W.1; price 3*s*. 6*d*.

Applications of Solar Energy

In *Solar Energy Technology: 1954–1959* (Library Association. Special Subject List No. 34. Pp. 32. London: Library Association, 1961. 3*s*. 6*d*.), T. Brimelow attempts to list all the significant literature on the subject published in Europe and the United States with the exception of items published in the popular and political periodicals of the U.S.S.R. The 329 items are arranged in sections which include: aero/space applications; air-conditioning; automotive engineering; biological science; electrical conversion; furnaces; heating; radio; refrigeration; water heating and water treatment. There are an author index and also a subject index, and a brief introduction by Dr. H. Heywood notes the salient features in present applications of solar energy.

Scientific Research in British Universities, 1960–61

Scientific Research in British Universities, 1960–61, issued by the Department of Scientific and Industrial Research, is an indispensable handbook for all those who endeavour to keep track of scientists in the United Kingdom and their fields of research (Pp. xiii + 513. London: H.M.S.O., 1961. 32*s*. 6*d*. net). The handbook describes the nature of research projects in progress, and indicates the scope of research carried out by various scientific departments and individual teams of investigators. Entries are made in alphabetical order of university, and subdivided into faculty and subject. Simple numerical headings of universities and subject allow ease of reference. Three indexes are provided, satisfying any search by name, subject heading or actual research.

Lady Tata Memorial Trust: Awards for 1961–62

The Trustees of the Lady Tata Memorial Trust, on the recommendation of the (European) Scientific Advisory Committee, have made the following awards for research on leukæmia and allied diseases in the academic year beginning October 1: *Grants for research expenses*, Dr. J. L. Amiel, Cancer and Radiopathology Research Centre, Paris; Dr. B. M. Braganca, Indian Cancer Research Centre, Bombay; Dr. G. W. Griffith, County Health Department, Anglesey; Dr. B. Lagerlof, Karolinska Institute, Stockholm; Dr. H. J. Woodliff, New Pathology Laboratory, Radcliffe Infirmary, Oxford. *Scholarship*, Dr. L. Chieco-Bianchi, Institute of Pathological Anatomy, Bari.

Awards in Radiological Research and Nuclear Medicine: James Picker Foundation

On behalf of the James Picker Foundation, the National Academy of Sciences—National Research Council is accepting applications for fellowships and grants in the field of radiology and nuclear medicine for the year 1962–63. Awards are made in four categories: (1) *Advanced Fellowships in Academic Radiology*. Completion of clinical training in radiology ordinarily will be a prerequisite, and the candidate must be prepared to devote a minimum of two years to course work in the basic sciences and to the application of the techniques and methods of these basic disciplines to radiological research. (2) *Post-doctoral Research Fellowships*, open to recent graduates who desire investigative experience and training in radiology or nuclear medicine. Applicants must hold the M.D., Ph.D. or Sc.D. degree or the equivalent. Awards are for a period of one year. (3) *Grants for Scholars*, to assist medical schools in supporting and developing junior staff members in radiology. Initial grants are made for two years. (4) *Grants-in-Aid of Research* are designed to encourage investigations in radiology and nuclear medicine offering promise of improvement in methods of diagnosis or treatment of disease. These grants may be made for two years. Applications for the fiscal year 1962–63 should be submitted by October 1, 1961, to the Committee on Radiology, National Academy of Sciences—National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D.C.

The Institute of Physics and the Physical Society

At the first annual general meeting of the amalgamated Institute of Physics and the Physical Society, held in London on July 4, the following were elected to office: *President*, Sir John Cockcroft; *Vice-Presidents*, Dr. V. E. Cosslett, Prof. R. W. Ditchburn, Dr. W. H. Taylor and Dr. J. Topping; *Honorary Treasurer*, Dr. J. Taylor; *Honorary Secretary*, Dr. C. G. Wynne. Eight ordinary members of Council were also elected. This, the first elected Council of the amalgamated body, is very nearly the same as the 'caretaker' Council which has been in office since the amalgamation of the Institute and Society on July 5, 1960.

University News: Leeds

The Foundation for Management Education has arranged for grants totalling £16,600 a year for seven years to be made available to the University of Leeds. This will make possible the strengthening of the present teaching in management studies for students

mainly in the Faculty of Technology; and the expansion of the work with residential and non-residential courses for upper and middle managers, and the establishment of a one-year postgraduate diploma course in industrial management. It will also enable the University to gain the assistance of a number of visiting lecturers, and to set up a number of scholarships and fellowships for research in the field of management and business studies.

The following appointments to lectureships have been announced: Dr. A. Chadwick (zoology); Dr. B. L. Shaw (inorganic and structural chemistry). Mr. F. P. G. Whitaker, at present lecturer in industrial management, has been appointed director of management studies in the Department of Economics and Commerce.

London

Dr. J. S. Webb, reader in applied geochemistry at the Imperial College of Science and Technology, has been appointed to the University chair of applied geochemistry tenable at that College. Dr. F. G. A. Stone, assistant professor at Harvard University, has been appointed to the University readership in inorganic chemistry tenable at Queen Mary College from July 1, 1962. The title of reader in biology in the University of London has been conferred on Dr. Agnes M. Hughes in respect of her post at the Royal Free Hospital School of Medicine.

Prof. J. N. P. Davies, professor of pathology at Makerere College, has been appointed to the University readership in morbid anatomy tenable at the Postgraduate Medical School of London. Mr. H. D. Ritchie, assistant director of the Surgical Unit at the London Hospital, has been appointed to the University readership in surgery tenable at the London Hospital Medical School.

Titles of reader in the University have been conferred on: Mr. R. W. Fearnhead (dental anatomy), in respect of his post at the London Hospital Medical College; Dr. T. G. Bonner (organic chemistry), in respect of his post at Royal Holloway College.

North Staffordshire

The University College of North Staffordshire, Keele, is to become Keele University in 1962. H.R.H. Princess Margaret, who is at present president of the College, has been proposed as the first chancellor of the new University.

The following appointments have been announced: H. Liebeck, to a lectureship in mathematics; Dr. M. Asgar Ali, to an Imperial Chemical Industries research fellowship; M. J. A. Smith, to be Mullard Research Fellow in physics; Dr. W. P. McCann, to be Leverhulme research officer.

Oxford

The first award of the J. H. Burn fellowship, at the Department of Pharmacology, for 1961–62 has been made to Mr. H. P. Rang.

Sheffield

The following have been appointed to readerships: Dr. L. Mirsky (pure mathematics); Dr. V. R. Pickles (physiology); Dr. F. A. Benson (electronics). G. Lederer has been appointed as a lecturer in pure mathematics. Dr. P. B. N. Neal is to be senior Research Fellow in mechanical engineering, and Mr. A. B. Hedley has been appointed to the Central Electricity Authority research fellowship in fuel technology.

Announcements

The David Anderson Berry Prize for 1961 of the Royal Society of Edinburgh has been awarded to: (*a*) Drs. H. B. Hewitt and C. W. Wilson (Westminster Hospital School of Medicine, London) for their quantitative estimation of the lethality of radiation on leukæmia cells *in vivo*; and (*b*) Dr. D. H. Sussdorf (research fellow in immunochemistry, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena) for his studies on the re-population of the spleen of X-irradiated rabbits by lymphoid cells from shielded regions.

Dr. J. F. Goodman has been appointed head of the Basic Research Department, Thomas Hedley and Co., Newcastle upon Tyne. Dr. Goodman joined Hedley as a research chemist in 1957, and in 1960 became head of the Physical and Analytical Group in the Basic Research Department.

Dr. Leo P. Vernon has been appointed as director of research of the Charles F. Kettering Laboratory in Yellow Springs, Ohio. Dr. Vernon, who is well known for his work on photosynthesis, is at present on sabbatical leave at the Nobel Institute, Stockholm, from his post as professor of chemistry at Brigham Young University. Dr. Vernon will assume his position at Kettering Laboratory on or about October 1.

The Plant Phenolics Group is holding a meeting on "Wood Extractives" at Royal Holloway College, Englefield Green, Surrey, during September 20–22. Further information can be obtained from the Honorary Secretary, B.L.M.R.A., Milton Park, Egham, Surrey.

"The Fishing Research Vessel" will be the major subject at a forum to be organized by the Food and Agriculture Organization in Tokyo during September 18–30. The meeting will also consider hydrographic survey ships, polar exploration vessels, hospital ships, lighthouse tenders, cable ships and non-magnetic ships. Further information can be obtained from the Food and Agriculture Organization, Rome.

The Systematics Association and the Challenger Society are arranging a symposium on "Speciation in the Sea", to be held at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, during September 27–29. Further information can be obtained from Norman Tebble, Zoological Secretary, Systematics Association, British Museum (Natural History), Cromwell Road, London, S.W.7.

An Advanced Study Institute on Underwater Acoustics is to be conducted during July 31–August 11 of this year in the Physics Department in the Imperial College of Science and Technology, South Kensington, London, S.W.7. Dr. Vernon M. Albers, chief scientist at the Ordnance Research Laboratory of the Pennsylvania State University, and Dr. R. W. B. Stephens, reader in acoustics at Imperial College, are co-chairmen of the Institute.

The Institute of Physics and the Physical Society is organizing a residential symposium on "Some Aspects of the Physics of Space Research" to be held at the Royal Military College of Science, Shrivenham, Swindon, Wiltshire, during September 20–22. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

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THE BRITISH SPACE DEVELOPMENT CO., LTD.

THE British Space Development Co., Ltd., has been formed in order to bring together a representative cross-section of that part of industry which is interested in the development and exploitation of space for commercial purposes and which believes that the use of space will, in the next two decades, become a major key to the continued prosperity of the United Kingdom and Commonwealth, politically and industrially. It is considered that a successful and economic entry into the new medium of space demands a unified effort on the part of British industry.

Briefly, the initial aims of the Company are as follows:

(*a*) To provide an organization to discuss with the Government and co-operate in any proposals that the Government may make in regard to a United Kingdom and Commonwealth space programme, which is either independent of European participation or which includes European participation.

(*b*) To provide the Government with an indication of the faith that British industry has in the exploitation of space and to show its readiness to play its part and to take its share of the risks as well as its share of the future benefits.

(*c*) To offer the Government an industrial body broadly based to act as an authority or agency to conduct research and development and to implement and operate a space programme and to consider and develop all the possible areas of exploitation of space.

(*d*) To provide those industries concerned with an organization to negotiate with other countries or foreign industrial organizations or consortia any matters concerning the commercial uses of space which are likely to have global implications, that is, communications, broadcasting, television, meteorological forecasting and navigational aids.

(*e*) To provide the greatest economy in the use of the limited number of technical personnel available in the country, particularly in the field of astrophysics and astronautical engineering, and to ensure that duplication of effort, which has been so prevalent in the United States and which has contributed to wasteful use of resources, is avoided in the United Kingdom.

(*f*) To encourage the provision of and to provide directly, in due course, facilities for the training of an adequate number of technical personnel in all those sciences associated with space research and the exploitation of space.

(*g*) To preserve for British industry a place in space and a proper share of the national and international benefits deriving from the development and use of space.

It is possible that the Company may develop into an international company, particularly as no enterprise in the world has as yet been envisaged where such a wide cross-section of industry has come together to pool its resources and effort in order to compete in this demanding, expensive but ultimately most lucrative new technical field with its limitless possibilities.

The British Space Development Co. now being formed will have an initial capital of £20,000. The following Companies will be the initial participants: Associated Electrical Industries, Associated Tele-Vision, British Insulated Callender's Cables, Decca Radar, Hawker Siddeley Aviation, Ltd., Pye, Rank Organization, Rolls-Royce, Plessey, Elliott Automation.

As one of its first major projects, the Technical Committee of the Company is now examining the technical and economic problems of the United Kingdom and Commonwealth satellite communications system which allows for integration with an American system.

Sir Bernard Lovell, professor of radio astronomy in the University of Manchester and director of the Nuffield Radio Astronomy Laboratories, Jodrell Bank, has been appointed scientific adviser to this newly formed Company, though he will retain his present position at Manchester and Jodrell Bank.

THE DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

THE report of the Council for Scientific and Industrial Research for the year 1960* records gross expenditure for the year ended March 31, 1960, of £12,008,536, compared with £10,059,376 in the previous year, and reduced to £10,342,691 by various receipts from industry and other sources for services rendered. Of the latter, £722,241 was from other Government Departments and £723,601 from industry, £613,592 in all being received by the National Physical Laboratory: the net increase on 1959 was £1,447,871. Grants to students amounted to £870,289, the number of research students in training being 2,377, of whom 942 (including 88 NATO awards) were new; advanced course studentships numbered 315 (of which 274 were new), first-year studentships 77, and research fellowships 75, of which 30 were held overseas and 45 in Great Britain, and 39 (including 21 NATO fellowships) were new.

Of the research students, 290 were in biology and biochemistry, 176 in chemical engineering, 747 in chemistry, 77 in electrical and 162 in other engineering, 143 in geology, 43 in the human sciences, 236 in mathematics and 503 in physics. Of the advanced-course students, 67 were in physics, 62 in mathematics, 20 in electrical and 42 in other engineering, 39 in geology, 28 in biology and biochemistry, 27 in chemical engineering and metallurgy, 25 in the human sciences and 5 in chemistry. All 77 first-year studentships were in chemistry, and chemistry (22), physics (21) and biology and biochemistry (18) claimed most of the research fellowships.

Grants for special researches totalled 525, of which 293 were new, amounting to £5,591,270; of these, 75

* Department of Scientific and Industrial Research. Report of the Research Council for the year 1960. Pp. 65. (Cmnd. 1365.) (London: H.M. Stationery Office, 1961.) 4*s.* net.

were in biology, 102 in chemistry, 37 in geology and geophysics, 3 in mathematics, 19 in astronomy, 65 in low-temperature work and investigations on the solid state, 60 (totalling £2,221,896) in nuclear physics, 90 in technology, 23 in the human sciences, and 24 (totalling £297,543) in space research. The report does not include the list of individual grants exceeding £1,000 or more, nor is the analysis of the research effort of the Department's research stations given in last year's report repeated.

Expenditure on the Warren Spring Laboratory was £402,854 gross, compared with £404,963 in 1959, and on the Torry Research Station, Tropical Products Institute and Government Chemist, which appear in these accounts for the first time, £177,860, £164,051 and £290,237, respectively. Otherwise, there are increases under all the separate heads of expenditure, the largest increases being in headquarters administration (£623,206 from £441,440); the National Physical Laboratory (£1,438,776 from £1,285,018); building research (£656,975 from £599,931); Geological Survey and Museum (£490,014 from £433,550); National Engineering Laboratory (£686,156 from £637,299); and road research (£718,056 from £618,763). Expenditure on fire research amounted to £158,441; on hydraulics research to £175,041; on radio research to £239,174; and on water pollution research to £137,100.

Annual grants to research associations amounted to £1,761,368 (£1,683,195 in 1959) and special grants to £17,823; £1,259,000 was contributed to the European Organization for Nuclear Research and £75,333 to NATO scientific schemes. Expenditure on headquarters overseas liaison increased from £113,929 to £132,684. Further progress is reported with the planned expansion of the staff, which rose by 3 per cent from 5,506 to 5,680 during the year, and it is estimated that, excluding stations transferred to or from the Department, the total staff has increased by just under 20 per cent during the past four years.

THE RADIO RESEARCH STATION, SLOUGH

THE Radio Research Station, Slough, gave visitors the opportunity of seeing some of its work during two open days on May 31 and June 1. Most of the Station's work is concerned with radio-wave propagation and with the fundamental properties of the ionosphere and the troposphere through which the waves travel. About half of the work is devoted to some aspect of experiments made on rockets and artificial satellites.

Among the exhibits concerned with the propagation of very-high-frequency waves through the troposphere was one which showed how the dielectric constant of the air had been measured continuously in an aeroplane. The measurement was made by determining the natural frequency of an open-ended electromagnetically resonant cavity. The measured distributions of dielectric constant were then related to certain aspects of radio wave propagation. Of other exhibits concerned with tropospheric propagation one showed a method for determining the azimuthal angle of arrival of waves propagated by tropospheric scatter from distances beyond the horizon, and another showed how the time-delays in multipath transmission over a distance of about 35 miles were being measured.

Equipment used for recording information about the ionosphere over Slough and over outstations at Falkland Islands, Singapore and Inverness was demonstrated. The results, together with those from other ionosphere observatories all over the world, are available for the use of all bona fide workers in the Station's World Data Centre. This is one of four set up in different countries by international agreement. One of the exhibits showed how the Research Station was making use of these recordings to investigate the nature of the ionosphere over the polar regions. Conditions of strong absorption (blackout) and of sporadic ionization at levels near 100 km. (*Es*) were shown to occur along spiral lines fixed in space relative to the Sun. When, as a result of the Earth's rotation, any given point on the Earth passes beneath one of these spirals, the appropriate phenomenon is observed. The two spirals, one for the blackout and the other for *Es*, curve in opposite senses, due, it is thought, to the fact that they are caused by the arrival in the atmosphere of particles of opposite sign. Another exhibit showed how the ionosphere over Halley Bay in Antarctica had varied throughout the International Geophysical Year.

The ionization produced in the ionosphere by the Sun's radiation varies throughout the day and the season as the angle of the Sun's rays, and possibly also the constitution of the atmosphere, changes. If changes caused by these two varying quantities could be eliminated the density of the ionization might be expected to be a measure of the intensity of the solar radiation. Methods for removing these variations were explained and the way in which the resulting 'ionospheric indices' varied with time were shown. One puzzling result was that the indices both for the *E* and *F* regions appear to have decreased steadily throughout the past 20 years even when the variations of the sunspot cycle have been removed.

The procedure was shown in which a digital computer was used to estimate the main parameters (height and peak electron content) of the *F* region from a knowledge of these quantities at a few key observing stations, and it was explained how this might help in the future for forecasting the nature of the ionosphere all over the world.

Once the world-wide *F* region is known, it is, in principle, possible to calculate the different rays by which waves will travel, on any frequency, from any one point to any other point on the Earth. The results of such calculations were compared with measurements of pulses received over the range of frequencies from senders in Malta and on Ascension Island. It is hoped that this kind of calculation, extended with the help of a digital computer, might lead to a simplified method for planning the frequencies used in point-to-point communications.

Another series of exhibits showed the work of the research station in the field of radio measurements. A resistive film inserted in a waveguide was used in an apparatus for the measurement of microwave power. This device had been compared satisfactorily with other standards from other laboratories and other countries.

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Realization of the danger of intense radio-wave fields has led, recently, to a need for their measurement. A method of calibrating field-strength meters for these measurements was shown in which the intense field was produced in the space between the two plates of a 'strip waveguide'. In this way a field of the necessary intensity was produced from a comparatively feeble source of power and was confined to the guide, where it was harmless.

Other exhibits showed what was being done in research concerned with rockets and artificial satellites. Observations of the Faraday and Doppler effects on the waves emitted from satellites had led to deductions about the total electron content of the ionosphere overhead. Observations of the orbits of satellites were being used to make deductions about the density of the upper atmosphere and its variation.

An experiment to investigate the electron density in the ionospheric *D* region is to be performed in a rocket fired from Watheroo in Australia. This experiment will be done in collaboration with the University of Sheffield. The apparatus in the rocket will measure the parameters of a low-frequency wave-field set up above a transmitter radiating waves from the ground on a frequency near 200 kc./s. Part of the equipment was shown.

The Research Station is to collaborate with Canada and the United States in an experiment designed to investigate the upper side of the ionosphere. In this, satellites designed and launched by those working in the other countries will carry equipment to sound the ionosphere from above. The part of the Radio Research Station will be to receive telemetry from these satellites at Slough, Falkland Islands and Singapore, and to compare the results of the 'topside' sounding with the results of sounding made at these three places from the ground.

The Research Station is responsible for manning and running a station of the 'Minitrack' system set up by the Americans on a world-wide basis for the tracking of artificial satellites and the reception of their telemetry. The equipment is provided on loan by the Americans and is installed at a field site in Winkfield, about eleven miles from the Research Station. The visitors were taken there to see it. Measurements of position and records of telemetry made at this Station are sent back to America and are also available for the use of workers on the Station.

A World Data Centre for Rockets and Satellites, one of three, is run by the Station which is also responsible for tracking the position of the more important satellites for the use of optical and radio observers in Britain.

THE ROYAL AIRCRAFT ESTABLISHMENT, FARNBOROUGH

THE open days held at the Royal Aircraft Establishment, Farnborough, during June 9–10 were the first for six years. (The annual Air Show is, of course, that of the Society of British Aircraft Constructors, who hire the airfield for the occasion.) They gave a vivid impression of an Establishment that can afford to remain silent about its 56 famous years of service to scientific aviation (except through the single exhibit of a flying *S.E.5a*, the fighter designed at Farnborough of which Britain built 5,000 in the First World War), presumably because the present is so packed with excitement and achievement in the Establishment's main fields of transport aircraft, military aircraft, space, weapons, aircraft equipment and general research.

A central display hall, transformed from an aircraft hangar into a pavilion with flowers and fountains, summed up the whole display. It set out in its first illustrated stand the organization of the Royal Aircraft Establishment, with its headquarters at Farnborough, its second ultra-modern research centre and airfield at Bedford, and its well-equipped ranges for weapon trials. Subsequent stands, each devoted to one of the six main fields just noted, showed by models and highly informative backcloths how the different kinds of scientist and engineer in the Establishment make their different contributions to each. For example, study of transport aircraft problems was stated to include work on 'cabin conditioning', 'engine intake design', 'metal fatigue' and 'communications engineering' as four out of some thirty separate contributions—and similarly with the other fields. Two further stands showed the impressive range of major equipment for experimentation which the Establishment possesses to help it do its work, and the large-scale workshops, drawing-office and aircraft-servicing effort that lies behind the scientific achievements.

Both this central hall, and the 108 specialized scientific or technological exhibits, showed an Establishment pushing vigorously forward, with all its extraordinarily diverse activities concentrated on a few clearly defined objectives—to get air transport safer, cheaper, faster, more comfortable, more reliable, to get for the Army, Royal Navy and Royal Air Force a complete range of aircraft and weapons which will use the latest scientific advances to meet economically the needs of war as they are now envisaged, to develop continually improved aircraft equipment for navigation, reconnaissance, communications, power generation, automatic control and even landing, and to ensure that in spite of a late start Britain reaps the benefits of operations in space. Inquiries showed that the extent of 'space' activity, not only on the development of launching vehicles based on *Blue Streak* but also on the technology of satellites themselves, with special emphasis on a satellite system for world-wide communications, would almost justify a re-christening as the 'Royal Aircraft and Spacecraft Establishment'.

In advanced scientific research the variety of activity was astounding. For example, supersonic and hypersonic aerodynamics were to be expected, but the strength of the Farnborough school of low-speed aerodynamic research was equally evident, and remains equally necessary. The complexity of its subject-matter was exemplified by a wind-tunnel investigation of the effect of ground proximity on the lift of an aircraft model with swivelling jets, after the manner of the *Hawker P.*1127 vertical-take-off strike aircraft. Equally the 'slender-delta' designs of supersonic airliner have demanded for the solution of

their landing and take-off problems a new aerodynamics, the extension of which into the unsteady 'gust case' was exemplified by an exciting experiment in which balsa-wood models were catapulted across a 24-ft. wide jet of air and their response in roll and yaw recorded by ciné-camera.

Another flourishing research school is in physics and chemistry of materials. The physical mechanisms of metal fatigue, including the details of crack development under cyclic loading, are now, in the main, scientifically determined, and are being rapidly related to the fatigue-life estimation of aircraft structures, showing, as one surprising result, that increase of temperature can sometimes extend fatigue life, because crack propagation in each cycle may halt sooner if plastic working is facilitated. Research on new materials has uncovered some impressive carbons with good strength and zero permeability, some new methods of construction in plastics reinforced with fibre-glass to give a strength-to-weight ratio 2·4 times better than 100 tons/in.^{2} steel, and a range of promising organic syntheses aimed at lubricants and polymers with improved high-temperature stability.

In the electronic field, an important new method of determination of the effective mass of charge carriers in semi-conductors was demonstrated, using observations of Faraday rotation, which was also shown modulating a beam of infra-red radiation at audio-frequencies. Promising circuits were exhibited, aimed at using semi-conductors to convert variable-frequency power from an aircraft's alternators to constant frequency. Research on aerials included the study of logarithmic-spiral forms at microwave frequencies, and some elegant work on corona breakdown on aircraft aerials at altitude. In the electronic equipment field, the miniaturized telemetry equipment, for transmitting pressures and other data to ground during weapon trials, stood out particularly.

Servocontrol is a well-known Farnborough activity. In the manned-aircraft field, it was interesting to see an aircraft being flown with all-electric signalling between the pilot's stick and the control surfaces. This is being used to facilitate a range of manœuvre demands. In the rocket field, the control of *Black Knight* by the swivelling of its two motors was demonstrated, together with equipment for stabilizing the attitude in space of the upper-atmosphere-research rocket *Skylark*. Equipment for studying the possible gyro-stabilization of a communications satellite so that its aerials will always point towards the centre of the Earth was also exhibited.

Research aimed at navigation and guidance was shown in impressive volume and quality. The propagation of very-low-frequency waves (of length around 20 km.) is now known to have remarkable stability as far as the antipodes even during magnetic storms, and research in a *Comet* aircraft flying from Farnborough has given good reasons to expect development of an airborne system for world-wide position determination within an error of at most 5 km. using only six ground transmitters. Inertial navigation has also been making extraordinarily rapid progress, with the Royal Aircraft Establishment again active, its longer-term researches being aimed, it appears, mainly at reduction in costs. Other titbits in this field were a tantalizing star-tracking demonstration and a glimpse of the future for high-speed computers in air traffic control.

It would be impossible to do justice to this huge exhibition in a short article, although a brief mention of the fascinating stands relating to water-drop or ice-crystal impact on aircraft, to new wind-tunnel techniques for aeroelastic research, and to rocket techniques for aerodynamic research at very high speeds, can scarcely be resisted. The days ended with experimental flying displays, showing among other things how the British army of the future may receive its supplies without parachutes, by low-level drops of equipment on special shock-absorbing skids, and how flight research is adding to our knowledge of the physical characteristics, and the effects on aircraft, of atmospheric turbulence.

Mr. M. J. Lighthill and his staff at Farnborough can be warmly congratulated, both on the story that they have had to tell and on the manner of its telling.

PETER SANDERSON

BRITISH BAKING INDUSTRIES RESEARCH ASSOCIATION

NEW LABORATORY WING

A NEW laboratory wing at the Baking Industries Research Station, Chorleywood, was opened on April 27 by Mr. Trevor Bowen, honorary president of Barkers of Kensington, and a former master of the Worshipful Company of Bakers.

In declaring the new north wing open, Mr. Trevor Bowen recalled the early days of the Research Association in 1947, when it had few members and a staff of only three, and, to indicate his appreciation of the services rendered by the Research Association, Mr. Bowen made a most generous gift of one thousand guineas as a contribution towards research.

The facilities of the new wing have been made essential by the growth in the Association's membership and activities. The work of the Association has outgrown the extra accommodation made available in 1951, when the present west wing was opened, and the need for additional and improved facilities has been a pressing one for some time.

The new accommodation consists of a two-storey wing running northwards from, and linked to, the original building of Chorleywood Lodge, designed to blend with the existing buildings and erected at a cost of approximately £40,000, including equipment.

The main feature of the ground-floor is the pilot-scale biscuit bakery and its associated laboratory. New pieces of equipment, built specially by members of the Research Association, are a model forced convection travelling oven by the Spooner Food Machinery Engineering Co., Ltd., and a De Vuurslag rotary biscuit cutter supplied by John Pelkman Engineering Co., Ltd. The biscuit section will thus have, virtually, a model biscuit plant which will enable the whole range of hard- and soft-dough biscuits to be studied. It is expected that this will enable previous work to be expanded on such problems of biscuit production as checking, or spontaneous cracking, in biscuits, on changes in biscuit dimensions

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in baking, and on the effect of various factors such as fat composition and storage conditions on the development of fat bloom on biscuits. The new equipment should also greatly facilitate the testing of biscuit flours and the development of standard procedures for this purpose. Sectioning and staining procedures have enabled a technique to be devised for investigating the starch, protein and fat distribution in biscuits and these investigations will be continued.

A new and well-equipped laboratory has been provided for the Organic Chemistry Section. This will give much-improved facilities for the work which has been in progress for some years on flour lipids, which is substantially supported by a grant from the United States Department of Agriculture. The work on lipids represents one facet of the attack on the problem of why flours differ in baking quality. Its immediate aims are to establish whether wheats of different varieties grown under identical conditions differ in lipid content, the influence, if any, of seasonal or environmental factors on the lipid composition of a single wheat variety and, finally, whether the lipid content or composition of a flour may be correlated with its baking properties. Also being studied are the fatty acid distribution in flour lipids, and methods for the separation of flour lipids in undegraded form.

These investigations, together with others in progress at the Research Station, will be greatly facilitated by the Buhler mill, which forms part of the equipment of the new Cereals Research Laboratory. This mill will provide much-needed facilities for milling wheat varieties on the site in connexion with the lipid project and other investigations, such as that of protein fractions being carried out in the new Physical Chemistry Laboratory. These provide another aspect of the problem of baking quality. Proteins of the gluten complex are being studied in the hope of establishing why wheats of similar protein content differ so markedly in this respect, and the work ultimately may lead to an understanding of the vast superiority of wheat protein over other cereal protein for bread-making. The present emphasis is on separation of gluten into its constituent proteins using starch-gel electrophoresis.

Also in the Physical Chemistry Laboratory are located the investigations into bread staling at present being pursued. These are concerned with those properties of wheat starch which influence the hardening of bread during the staling process with particular emphasis on the role of anti-staling agents. These agents increase the initial softness of bread and reduce the rate at which it hardens on ageing. Investigations of the interaction of anti-staling agents and starch are in progress in order to elucidate the basic mechanism of these effects.

An instrument and balance room has been provided on the first floor to house the more elaborate equipment used by all sections on different occasions.

At the southern end of the first floor the new Microbiology Laboratory extends the width of the building. There are a separate room for sterilization and another with filtered air-intake suitable for sterile techniques. The laboratory is concerned with all aspects of the microbiological deterioration of baked goods, but at present the work is mainly concerned with factors affecting mould growth in baked products, such as the effect of humidity, sugar concentration, and the degree of acidity or alkalinity of the mix. The use of mould inhibitors, such as propionic acid and its salts, as ingredients in bread and cake is also being studied. It is expected that legislation will shortly be passed to allow the use of these compounds, and the most effective ways of employing them and of overcoming side-effects, such as a reduction in bread volume, are being worked out.

Further facilities in the north wing are a combined office and laboratory for the Deputy-Director of Research, and another for the head of the Member Services Division, who is also responsible for applied research projects.

There is also office accommodation for two members of the newly formed Scientific Liaison Service set up to complement the work of the Industrial Liaison Service, which is staffed by trained bakers. The purpose of this Scientific Liaison Service (at present staffed by a chemist and an engineer) is to promote the application of the results of scientific research in the baking industry and to assess those problems in industry which require some research for their solution. These objectives are being attained primarily by personal visits and discussion, but also by lectures, demonstrations and collaborative experiments. A mobile laboratory has been equipped to take scientific apparatus to members' bakeries and has already proved its worth.

G. A. H. Elton

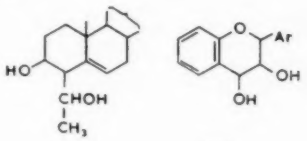
STEREOCHEMISTRY OF NATURAL PHENOLIC COMPOUNDS

THE aim of the Plant Phenolics Group symposium, held at the University of Bristol on December 16, was to discuss recent developments and current problems concerning the stereochemistry of natural phenols against the background of available methods for determining the stereochemistry of molecules.

Dr. B. R. Brown (University of Oxford) dealt with the determination of relative stereochemistry.

The methods available for the determination of relative stereochemistry were illustrated by reference to the conformationally rigid, steroidal diol system (I) and to the conformationally non-rigid flavan-3,4-diol system (II). Infra-red measurements of C—O stretching frequencies enable reliable conclusions to be reached about the stereochemistry of the 3-hydroxyl group in (I) but not about the hydroxyl groups in (II), because of interference from aromatic vibration frequencies. Similarly, infra-red studies of hydrogen-bonding in (I) lead to reliable conclusions, but those from (II) do not, because of the conformational lability of the system. Ring formation to give carbonates or *iso*propylidene derivatives is diagnostic with (I) but not with (II), the *cis* and *trans* isomers of which both form cyclic compounds. In view of this, measurements of the rates of cleavage of flavan-3,4-diols with lead tetraacetate is in progress, a method which enables the *cis* and *trans* isomers of cyclohexane and other cyclic 1,2-diols to be differentiated. The method of molecular rotation differences has been applied with success to the steroids (Barton) and to the catechins (Freudenberg) and more recently by

Weinges[1] to correlate naturally occurring catechins, flavanonols, and flavan-3,4-diols (leucoanthocyanidins). The application of enzymic methods to sugars is well known and a recent investigation by Bokadia, Brown and Somerfield[2] indicates that it may have value when applied to polyphenols of the catechin type.



(I) (II)

Prof. W. Klyne (Westfield College, University of London) described the determination of absolute stereochemistry.

The determination of the structure of an asymmetric natural product should not be considered as complete until its absolute configuration is known. The principal methods, with examples taken from different fields of organic chemistry, and chiefly from recent literature, are as follows:

(1) Chemical reactions, without displacement at the asymmetric centre, concerned: (*a*) degradations, for example, catechins (Hardegger *et al.*), cadinene (Herout and Šorm), and methymycin (Djerassi); (*b*) syntheses, for example, the conversion of representatives of the yohimbine–corynantheine–ajmalicine alkaloids and cinchona alkaloids into a common intermediate (Wenkert).

(2) Chemical reactions, with displacement at the asymmetric centre, concerned, for example, the classical studies of Ingold, Hughes and their colleagues on the steric consequences of nucleophilic substitution. Arguments by analogy include the work of Balenovic on β-aminobutyric acid, and of Witkop and of Sicher on ephedrine.

(3) Asymmetric synthesis (Prelog and Cram), for example, the catechins (Birch) and the diphenic acids (Mislow, also Berson).

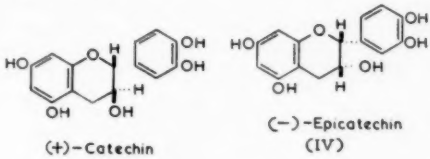
(4) Formation of quasi-racemates (Fredga), for example, the correlation of (+)-methylsuccinic acid and (−)-thiolsuccinic acid, which gave the first link between branched-chain compounds and the glyceraldehyde series.

(5) X-ray crystallographic methods do not, in general, distinguish between enantiomers, but an ingenious technique due to Bijvoet (Utrecht) which uses X-radiation of a wave-length which is partly absorbed by an atom in the crystal under examination permits a solution of the phase problem and determines the absolute configuration of the structure. This method, first applied to sodium rubidium tartrate, showed that by a fortunate chance the Fischer convention for glyceraldehyde is correct.

(6) Optical rotation evidence: (*a*) rotatory dispersion, (*b*) monochromatic rotations. All structural and stereochemical arguments which make use of optical rotations are based on analogy, and the usefulness of such arguments depends on the care with which analogies have been chosen. Recent instrumental developments have permitted the measurement of complete rotatory dispersion curves (that is, rotation : wave-length curves). Djerassi has found that "typical features of dispersion curves of alicyclic monoketones are on the whole only a reflexion of the immediate structural and stereochemical environment around that particular carbonyl group". A notable application of this principle was to the problem of the absolute configuration of the 'abnormal' diterpene, eperuic acid (Djerassi, F. E. King).

Dr. J. W. Clark-Lewis dealt with the stereochemistry of flavan derivatives.

The absolute configurations of the catechins were determined in 1957 and although the 2*R* : 3*S*-configuration of (+)-catechin (III) was unequivocally established, it was not until 1960 that the 2*R* : 3*R*-configuration of (−)-epicatechin (IV) received confirmation. The occasion of this symposium was therefore timely for reviewing the evidence for the configurations of the catechins. The diagnostic reaction for *cis*-flavan-3-ols is the elimination of toluene-*p*-sulphonic acid from tosyl esters, and for *trans*-flavan-3-ols is their rearrangement to 2-alkoxy-*iso*flavans; these two reactions have been used to characterize new catechins obtained by hydrogenolysis of leucoanthocyanidins and dihydroflavonols. Relative configurations of the catechins follow from the formation of enantiomeric 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)-propan-2-ols after reduction of (+)-catechin and (−)-epicatechin methyl ethers with sodium and liquid ammonia. Reduction with lithium aluminium hydride and aluminium chloride mixture is accompanied by a 1,2-rearrangement and leads, after methylation, to formation of (−)-2-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)-propan-1-ol from both diastereoisomers. It was shown that this rearrangement confirms the relative configurations of the catechins, and that it proceeds by benzyl-group migration. The absolute configurations of (+)-catechin and (−)-epicatechin are known from ozonolyses and from the application of Prelog's atrolactic acid method. The stereochemistry of several naturally occurring flavanoids can now be correlated with the stereochemistry of the corresponding catechins obtained by hydrogenolysis.



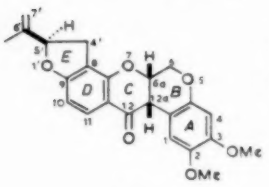
(+)-Catechin (III)

(−)-Epicatechin (IV)

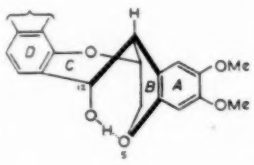
Dr. L. Crombie (King's College, University of London) described the absolute configuration of rotenone and its derivatives.

Work by Prof. G. Buchi (Massachussets Institute of Technology) and Dr. L. Crombie *et al.* has led to complete stereochemical proposals for the natural insecticide rotenone (V). By ozonolysis and oxidation the 5′ centre was extracted from the rotenone degradation product (−)-dihydrotubaic acid as (+)-3-hydroxy-4-methylpentanoic acid. The latter was related, by a series of transformations, to D-glyceraldehyde. Ozonolysis of the enol-acetate of 6′,7′-dihydrorotenone yielded the 6*a* centre as (−)-glyceric acid and the absolute configuration at 6*a* and 5′ is thus established. The B/C ring fusion of rotenone is the thermodynamically favoured form and this was shown to be *cis* by study of the stereoisomeric 12*a*-hydroxy*iso*rotenones, by hydrogenation evidence, and by infra-red methods. For example, the borohydride-reduction products of rotenone and its relatives show a hydrogen bond between the new 12-hydroxyl and the 5-oxygen which is possible only

with a *cis*-fusion (cf. VI). The complete configuration of rotenone is thus (*6aS*, *12aS*, *5'R*) as in (V).



(V)



VI

Dr. W. D. Ollis, University of Bristol, spoke on the relative configuration of the rotenoids.

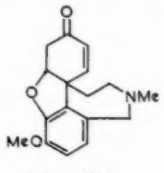
In collaboration with Prof. Carl Djerassi (Stanford University), it has been shown that the absolute stereochemistry of the eight natural rotenoids is closely related to that of rotenone (V). They all have the same relative stereochemistry at C_{6a} and C_{12a}; sumatrol and rotenone also have the same stereochemistry at $C_{5'}$. These stereochemical relationships have been established by optical rotatory dispersion studies on natural rotenoids and on derivatives of rotenone which were selected in order to assess the contributions of C_{6a}, C_{12a} and $C_{5'}$.

This work is complementary to that of Crombie and Büchi described in the preceding paper on the determination of the absolute stereochemistry of rotenone and indicate that the biosynthesis of the rotenoids is highly stereoselective. The stereochemical relationship between flavanoids, *iso*flavanoids and rotenoids, and possible biosynthetic implications were discussed.

Prof. D. H. R. Barton (Imperial College of Science and Technology, University of London) described alkaloid synthesis.

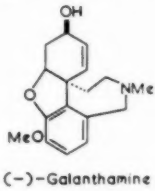
The biogenesis of many plant products may proceed through the oxidative coupling of phenolic precursors. In recent years several successful chemical syntheses have been based on this biogenetic idea; for example, oxidation of methylphloracetophenone and dehydration of the product gave the mould metabolite, usnic acid. Certain alkaloids may also be derived in principle by oxidation of suitable phenolic intermediates, the morphine group providing a particularly attractive example.

The structure of the Amaryllidaceae alkaloid galanthamine (VIII) was predicted on biogenetic grounds, and a chemical synthesis has now been completed. The hypothetical phenolic precursor was synthesized and its oxidation by a variety of reagents studied. Polymerization occurred readily but it was eventually possible to isolate the desired α,β-unsaturated ketone, narwedine (VII), in low yield. Reduction of narwedine gave a mixture of epimeric alcohols one of which was shown to be racemic galanthamine. A comparison of the physical properties of galanthamine and epigalanthamine showed that the hydroxyl group in galanthamine is hydrogen bonded to the ether ring oxygen atom, thus establishing the stereochemistry of these alkaloids. Narwedine racemized readily in hydroxylic solvents but crystallization of a mixture of (±)-narwedine (VII) and (−)-galanthamine (VIII) from ethanol gave (+)-narwedine. A study of this unusual phenomenon has led to the resolution of narwedine and galanthamine.



Narwedine

(VII)



(−)-Galanthamine

(VIII)

The biogenesis of Amaryllidaceae alkaloids is at present being investigated using precursors labelled with carbon-14. Preliminary experiments show that both tyrosine and the phenolic intermediate are incorporated into galanthamine in King Alfred daffodils.

Ben Brown

[1] *Annalen*, **627**, 229 (1959).

[2] *Proc. Chem. Soc.*, 280 (1960), and unpublished work.

INTERNATIONAL CONVENTION ON STARCH

THE annual International Starch Conference was once again held at Detmold, during April 26–28, and was attended by nearly 300 chemists from 25 nations. Twenty-five papers were presented to the meeting and they were classified as follows: (1) starch research; (2) industrial research; (3) derivatives.

A series of beautiful colour microphotographs were exhibited by Dr. A. H. Czaja (Aachen), showing the manner in which starch granules gelatinize in water. When suspended in a strong salt solution in the presence of I_2–KI the blue amylose fraction was seen to bleed out of the granule, leaving the violet amylopectin behind in the intact structure. No such bleeding of amylose was observed when the reaction was carried out in iodine-saturated water. Here the whole granule was stained violet throughout. When the granule was then damaged slightly by rubbing with a glass rod, the amylose blue colour was seen to develop. Potato, maize and rice starch behaved similarly.

When using a fluorescence dye, small starch granules were seen to fluoresce strongly (red) while large granules showed a weak green colour. No explanation of this behaviour, which is opposite to that expected, is yet known.

Acridine orange showed that granules of wheat starch were surrounded by a thin film of protein.

The renewed interest in the degradation of starch by physical means was discussed by S. Augustat (Potsdam-Rehbruecke), who had used a vibratory ball mill. In this machine the vessel does not rotate, but contains a large number of balls which are agitated with a frequency of 1,420 vibrations per min. The amplitude of the vibrations is about 2 mm., and the powder in the mill is kept in circulation by the air-stream generated by the balls. After treating potato starch in such a mill for 5 hr. complete solubility in cold water was achieved together with a strong rise in reducing power. It was considered that this process may possibly have a future practical application.

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The quantitative estimation of ether and ester groups in starch derivatives was described by Dr. R. J. van der Bij (Foxhol, Holland). A thin film of starch was cast on 'Pliofilm' and after removal the film was pressed between plates of rock salt. The estimation was carried out with the infra-red spectroscope, which gave a characteristic absorption band at 5·8μ. The intensity of the absorption was proportional to the degree of substitution, but a correction for the thickness of film had to be made.

The problems which arise through automation in the starch-producing industry were described by H. Runge (Hamburg). The starch industry is peculiar in that certain processes do not lend themselves to automation, so that an operative may be called on to perform his work in the conventional manner as well as by instrumentation. The main problem encountered in this industry was the education of a new generation of operatives who were able and willing to perform their tasks in this way.

A. Malyjev (Central Research Institute for the Starch and Starch-syrup Industry, Moscow) explained the use of the dextrose–sodium chloride double salt to facilitate the crystallization of dextrose in factories in the U.S.S.R. Dextrose syrup at 56° Brix was treated with 0·2 per cent sodium chloride (w/w) at 20° C. Crystallization was completed after 8 hr., and it was found that this was a much shorter period than experienced when crystallizing dextrose in the usual manner. The crystals obtained were larger and the yield (85 per cent) higher than normal. After crystallization the double salt was filtered off and decomposed in a syrup of 22° Brix, which had been cooled to 10° C.

The effect of certain cations on starch was discussed by G. Tegge and W. Kempf (Detmold) and Dr. S. Winkler (Berlin). Calcium and magnesium ions (such as occur in hard water) were found to lower the viscosity of starch pastes to a greater degree than sodium or potassium ions. It was maintained that the phosphoric acid ester of starch forms salts with these cations. It was claimed that the only true viscosity value could be obtained by having the ester in the free-acid form (called hydrogen starch). This form of starch was produced by washing the native, ungelatinized starch with $N/100$ hydrochloric acid for 10 min., followed by washing with distilled water for half an hour to remove the acid. Hydrogen starch was said to be unstable owing to slow auto-hydrolysis.

K. Pasirouk (Central Research Institute for Starch and Starch-syrup Industry, Moscow) described a new potato washing machine in use in Russian starch factories. Potatoes were freed from sand and grit by being shaken violently at 3,000 cycles/min. in a long stationary cylinder, through which a swift current of water passed. Potatoes vibrated with an amplitude of 5 mm. The machine dealt with 200 tons a day, each charge being 470 kgm.

A full account of the papers outlined above and of the remaining papers, together with the discussions which followed in each case, will be published in the journal *Die Stärke* in due course. E. Dux

THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

IT is not always realized that the United Kingdom has one of the largest dairy industries in the world. Milk production in this country now exceeds 2,000 million gallons a year, and the milk has a value to the consumer of about £450 million, an amount of production which far exceeds that of many other well-known dairying countries such as New Zealand, Denmark and Holland. This vast and progressive British industry is continually increasing in efficiency, and much of the progress being made is directly attributable to the work done at the National Institute for Research in Dairying, the annual report of which for 1960 is now available*.

The Institute has grown greatly since the War. It now consists of ten main research departments, and has a staff of about 400 of whom some 30 per cent are members of the scientific and experimental officer grades.

The scope of the Institute's research programme is very great, ranging, as the report shows, from a study of many widely differing practical problems such as the use of slatted floors in the housing of dairy cattle and the types of chemical agents that can be approved for cleaning dairy equipment to basic scientific studies such as those of the Physiology Department on the pituitary gland and lactation, the biochemical properties of mammary gland mitochondria and the isolation, chemical structure and biological properties of the œstrogen mircestrol.

No more than one or two of the many investigations now in progress can be referred to here. One particularly interesting example is concerned with the all-important question of the composition of milk on which, of course, the nutritive value of milk and milk products depends. It is now well known that much of the dietary energy absorbed by ruminants is absorbed in the form of the lower fatty acids which are produced in the rumen in different proportions from different types of feeding-stuffs. Collaborative work between two of the Institute's main research departments has shown that when isocaloric amounts of the lower fatty acids are given separately by ruminal infusion to lactating cows acetic acid increases not only the milk yield but also the fat percentage in the milk and the yields of fat, protein and lactose, whereas propionic acid has no effect on milk yield but increases the percentage of protein and decreases the percentage of fat. Butyric and lactic acids have no effect on milk yield but butyric acid increases fat percentage and lactic acid increases protein percentage. This type of information is already leading to a much better understanding of the factors that affect the composition of milk, and when further extended it should make it much easier than it is to-day to influence the composition of cows' milk within certain limits by suitable changes in the rations fed to the cows, a development which could be of far-reaching significance in the dairy industry.

Some of the other outstanding advances that are being made depend on the application of relatively new scientific methods to dairy problems. Work is being done, for example, on the differentiation and recognition of various strains and species of bacteria by paper chromatographic procedures, and the method is being applied in a detailed study of the

* National Institute for Research in Dairying. Report 1960. Pp. 164. (Shinfield: National Institute for Research in Dairying: University of Reading, 1961.)

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bacterial flora of cheese. Then again gas chromatography is being applied to determine the volatile components of milk, and this may ultimately provide the basis of a rapid and reliable creamery platform test of milk quality. In another example a spectroturbimetric method has been devised which enables the mean fat globule diameter of homogenized milk to be rapidly determined. Indeed, it is abundantly clear from the report that every effort is being made to apply the most up-to-date scientific knowledge, methods and equipment to the art and practice of dairying in all its branches.

The report includes a list which gives the titles and authors' names of the 165 papers which were published by members of the staff between October 1959 and September 1960. The Institute is to be congratulated on the variety, amount and high quality of the work reviewed in its report. J. A. B. Smith

FISHING IN MADAGASCAR

Within the last decade, the fish *Tilapia melanopleura* has brought about a radical change in the diet and social habits of a large proportion of the population of Madagascar. Fish supplements the otherwise protein-deficient diet of the islanders, and fishing has become a useful and pleasant occupation practised every day by whole families.

The fish, along with other species of *Tilapia*, was first introduced by the Government ten years ago. The aim was to provide a source of much-needed protein to balance the Malgache menu of rice and manioc, fruit and a little honey, enlivened only occasionally by a small amount of meat or fish.

The *Tilapia* soon established itself in the rivers and lakes and multiplied rapidly. Fishing became popular. Encouraged by the success of this initial venture, the Government was stimulated to extend the output of fish still further by creating fishponds in the central part of the island where there is abundant water. Government agents went from village to village showing the inhabitants how to construct the ponds. By 1958 there were 40,000 village ponds; to-day there are 80,000, providing nourishing meals where formerly fish was seldom eaten.

A recent survey by Dr. Rudolph Kreuzer, Food and Agricultural Organization, Fisheries Division expert, shows that the average catch per adult is about 11 lb. of small fish each day, and even a small child can catch 4 lb. (*Sci. afrique*, **23**; February 1961). Not surprisingly, the people who live near the rivers and lakes eat more fish daily than Europeans do in a week. But the quantities caught are related only to the fisherman's needs. There is still malnutrition in the dry areas of the island. Before this can be eliminated, it will be necessary to develop a fishing industry and overcome the distribution problems inevitable in the hot African climate.

A government pilot station on Lake Alaotra is already successfully producing smoked fish and fish meal. Dr. Kreuzer's investigations have shown that drying and salting would also be successful in the fishing villages along the western coast. There, shielded by a coral reef, a primitive technique, closer to hunting than fishing, is practised. First, a flotilla of outrigger canoes forms a semi-circle near the reef. By beating the paddles on the water, the men drive the fish inshore. Then the fishermen leap overboard to spear the catch in the water. Nets are used only to prevent the quarry escaping seawards.

Better methods of fishing and the development of economic methods of processing the catch would help towards correcting dietary deficiency where it still occurs. But formidable transport problems would still have to be solved, for dense bush isolates the west coast fishing villages from the rest of the island.

TERRESTRIAL EXOSPHERE AT HEIGHTS OF 1,000—1,700 km.

By MAX RÖMER

Sternwarte der Universität, Bonn

The successful launching of the satellite iota 1, 1960 (*Echo* I), with its extremely small ratio of mass to area $m./A. = 10^{-2}$ gm./cm.^{2} first offered the opportunity of deriving atmospheric densities above a height of 1,000 km. For the time-interval August 12–December 19, 1960, it was possible to determine the densities for the heights 1,700 km. down to 1,000 km. Considering the large amplitude of the diurnal effect[1] and the relatively small eccentricity of the orbit it is impossible to use the density scale height H_ρ in the well-known air density formula by D. G. King-Hele, G. E. Cook and D. M. C. Walker[2]. The acceleration of a satellite is caused by the actual air density along the orbit. Therefore the 'individual orbital scale height' H characterizing a linear approximation to the logarithmic density function in the individual orbit was calculated[3]. For this calculation a model of atmospheric densities by H. K. Paetzold[4] was used augmented with the diurnal variation derived by W. Priester, H. A. Martin and K. Kramp[1]. The knowledge of the exact shape of the auxiliary model is not important because in the density formula only $\sqrt{H}$ is used. The computed densities hold for the orbital points of highest density, that is, for 14 hr. true local time.

The choice of the drag coefficient was discussed in detail[3] including the interaction of the charged satellite with the ionized component of the exosphere, and $C_D = 2{\cdot}5$ was adopted as a reasonable value[5]. According to R. W. Parkinson, H. M. Jones and I. I. Shapiro all orbital elements are disturbed by the solar radiation pressure during one revolution[6]. For orbits of small eccentricity the secular perturbation of the semi-major axis is very small. But for the calculation of air density by means of satellite accelerations all periodic variations of the orbital energy are important, the period of which is greater than the mean distance between consecutive orbital data. Considering the unequal time-intervals of acceleration and deceleration due to the non-uniform orbital

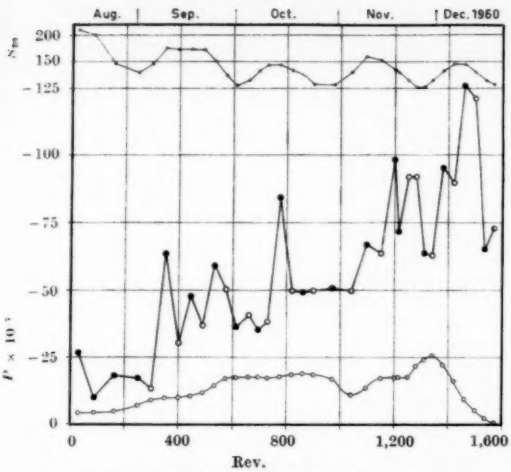

Fig. 1. Observed acceleration, acceleration due to solar radiation pressure and flux of solar 20-cm. radiation. The big circles of the curve in the middle of the diagram show the observed accelerations $P_{obs.}$ of satellite *Echo* I. According to the different weight, mean values $\Delta P/\Delta t$ are represented by full circles and less weighted values $\Delta P/\Delta t$ of the Bulletins of the National Space Surveillance Control Center, Bedford, Mass., by open circles. Accelerations due to solar radiation pressure P_{rp} are given by the small circles of the lower curve. The difference $P_{obs.} - P_{rp}$ yields the acceleration caused by aerodynamic drag. The flux of solar 20-cm. radiation S_{20} is shown in units 10^{-22} W./m.^{2} c./s. by the upper curve

velocity an approximate formula for the periodic perturbation of the period caused by solar radiation pressure (*rp*) was derived in co-operation with W. Priester:

$$\dot{P}_{rp} \simeq \frac{3 \times 8{\cdot}64 \times 10^4 \times P}{\pi} \cdot \frac{K \times \cos\theta_{\odot} \times e \times \sin(\omega - u_{\odot})}{m \times v} (\sin \Delta u_s - 2) \quad (1)$$

where P is period in days, $K = 330$ dyne, the product of area and radiation pressure if $S = 2{\cdot}0$ cal./cm.^{2} min. $= 1{\cdot}4 \times 10^6$ erg/cm.^{2}/sec. is adopted for the total flux of solar radiation, e is orbital eccentricity, v orbital velocity in cm./s., m mass of the satellite in gm., $\theta_{\odot}$ the angle between the orbital plane and the line Sun–Earth, ω argument of perigee, $u_{\odot}$ angle of the Sun in the orbit measured from the ascending node and Δu_s is half of the angle of the Earth's shadow in the orbit. Fig. 1 shows that the acceleration due to solar radiation pressure cannot be neglected in the analysis of the observed accelerations of *Echo* I.

For the calculation of atmospheric densities the difference of the observed acceleration $\dot{P}_{obs.}$ and the acceleration due to solar radiation pressure $\dot{P}_{rp}$ was used. These densities were corrected to a flux of solar 20-cm. radiation $S = 170$ (unit 10^{-22} W./m.^{2}c./s.) using the standard reduction formula (ref. 7). The correlation of the observed density $\rho_{obs.}$ with the flux of solar decimetre radiation S was checked and the best representation is:

$$\rho_{obs} \propto S^{m(h)} \quad (2)$$

where $m(h) \simeq 2$ for altitudes 1,000–1,700 km. The calculated densities ρ_{170} for the standard flux $S = 170$ drawn in a (log ρ, h)-diagram (Fig. 2) show a variation which can be explained by an 'annual' effect first found by H. K. Paetzold. The densities derived from *Echo* I by H. K. Paetzold[4,5], who uses a similar method which takes account of the large diurnal variation, are consistent within the limit of error if the influence of solar radiation pressure on the period is neglected. The values ρ_{170} were used by H. A. Martin and W. Priester[9] for expanding the density model (Bonn University Observatory model)[1] to 1,600 km. above the Earth ellipsoid.

Table 1. Physical Parameters of the Terrestrial Exosphere

h 10^3 cm.	g cm./s.^{2}	log ρ gm./cm.^{3}	H_ρ 10^3 cm.	log p dyne/cm.^{2}	H_p 10^3 cm.	T/M °K.	M for $T = 1690$ °K.
600	819·5	0·140 − 15	111	0·142 − 5	123	121	14·0
700	796·5	0·770 − 16	120	0·806 − 6	136	131	12·7
800	774·5	0·427	132	0·504	154	144	11·3
900	753·4	0·115	145	0·252	182	165	9·7
1,000	733·1	0·818 − 17	160	0·042	228	201	8·1
1,100	713·6	0·555	179	0·896 − 7	307	264	6·5
1,200	694·9	0·330	205	0·772	398	333	5·2
1,300	676·9	0·135	237	0·677	515	419	4·1
1,400	659·6	0·970 − 18	281	0·600	647	513	3·3
1,500	642·3	0·831	333	0·539	795	614	2·7
1,600	626·9	0·712	399	0·490	957	721	2·3
1,700	611·5	0·609	484	0·448	1,129	830	2·0

If local thermodynamic equilibrium holds and the velocity distribution is Maxwellian[10] the connexion of the equation of state for ideal gases and hydrostatic equilibrium yields the differential equation:

$$\frac{1}{\rho} \times \frac{\mathrm{d}\rho}{\mathrm{d}h} = -\frac{m \times g}{k \times T} - \frac{1}{T} \times \frac{\mathrm{d}T}{\mathrm{d}h} + \frac{1}{m} \times \frac{\mathrm{d}m}{\mathrm{d}h} \quad (3)$$

where m is mean mass of particles, k Boltzmann's constant, T temperature and g acceleration due to gravity. If the exosphere is assumed to be isothermal above the critical height h_c, the gradient of temperature vanishes and equation (3) can be solved numerically with an assumed value of the mean molecular weight M and the temperature T at h_c as initial condition. The integration is stopped when the mean molecular weight begins to increase with increasing height and when a lower limit of $M = 1{\cdot}5$ is reached. The distributions of mean molecular weight passing between these two limits from the critical height up to 1,700 km. determine the temperature and the

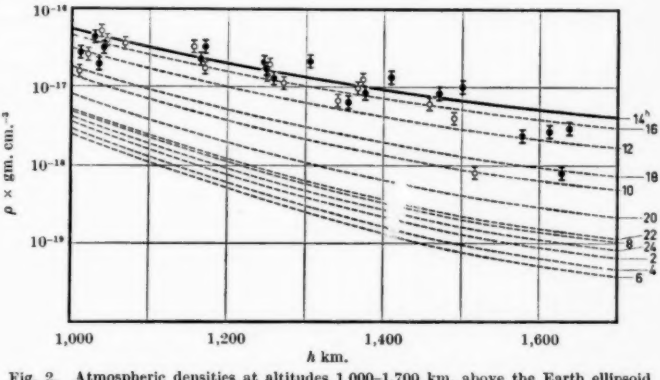

Fig. 2. Atmospheric densities at altitudes 1,000–1,700 km. above the Earth ellipsoid derived from satellite *Echo* I. The acceleration due to solar radiation pressure has been taken into account. The calculated densities (mean values represented by filled symbols, less weighted values by open symbols) hold for 14 hr. true local time and are corrected to mean solar activity ($S_{20} = 170 \cdot 10^{-22}$ W./m.^{2} c./s.). The 'fan shape' density model is the auxiliary model used for the calculation of the 'individual orbital scale height' H. The parameter of the curve gives the true local time. The time of year belonging to the densities is August 15, 1960, at $h = 1{,}640$ km. and December 18, 1960, at $h \simeq 1{,}000$ km.

lapse-rate of M in the exosphere. The temperature needed for the interpretation of the observed density distribution increases with increasing values of M at the critical height. For mean molecular weights of $M = 14, 16, 18$ at $h_c = 600$ km. the temperatures are 1,675° K., 1,900° K, 2,175° K. respectively.

One of the possible extrapolations of the density scale height $H_\rho = -\frac{dh}{d \ln \rho}$ leads to an isothermal exosphere with M = const. above 2,100 km. and to a pressure of $2{\cdot}02 \times 10^{-7}$ dynes/cm.^{2} at 2,100 km. The distribution of pressure can be calculated by:

$$p(h) = p(2{,}100 \text{ km.}) + \int_h^{2{,}100 \text{ km.}} \rho \times g \times dh \quad (4)$$

The important physical parameters of the terrestrial exosphere are given in Table 1: acceleration due to gravity g, density ρ, density scale height H_ρ, pressure p, pressure scale height $H_p = \frac{p}{\rho \times g} = \frac{R \times T}{M \times g}$ and the lower limit of mean molecular weight M for $T =$ 1,690° K. according to the H_ρ-distribution of the Bonn model. By means of the ratio T/M the mean molecular weight curve of an isothermal exosphere can be calculated for various temperatures. The unexpected small lapse-rate of the atmospheric density above 1,000 km. yields a rapid decrease of the mean molecular weight, that is, a rapid increase of hydrogen in the number density. For a detailed discussion of the problem see ref. 3.

I am grateful to Dr. W. Priester, who suggested this problem, for his co-operation and numerous discussions. Furthermore, I wish to thank the National Space Surveillance Control Center, Bedford, Mass., for supplying the orbital elements and the Heinrich-Hertz-Institut, Berlin-Adlershof, for the continuous dispatch of 20-cm. data prior to publication.

Note added in proof. According to recent investigations the orbital period of a satellite, which does not enter the Earth's shadow during one revolution, is not altered by solar radiation pressure. However, a comparison between the results plotted in Fig. 1 and the findings of R. W. Bryant (private communication) and of P. E. Zadunaisky *et al.* (*Smithsonian Astrophys. Obs. Spec. Rep. No. 61*, 1961) shows that formula (1) is a good approximation for the time-interval covered.

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[2] Cook, G. E., King-Hele, D. G., and Walker, D. M. C., *Proc. Roy. Soc.*, A, **257**, 224 (1960).

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USE OF THE ROTATING HANGING MERCURY DROP IN ANALYSIS

Anodic Stripping using a Rotating Mercury Drop

By Dr. J. J. ENGELSMAN and A. M. J. M. CLAASSENS

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A HANGING mercury drop has been used by several authors, for example, DeMars and Shain[1] and Kemula and Kublik[2], for the analysis of metals forming amalgams with mercury after electrolysis. Usually the metals in the amalgam are anodically dissolved by applying a potential which varies linearly with time. On the basis of preliminary calculations involving the diffusion conditions in and around the mercury drop, we were led to believe that the time constant of the dissolution process should be of the order of a few seconds. Consequently, it should be possible to obtain separate dissolution peaks for each of the components of a multicomponent drop of amalgam, using a rate of voltage scanning such as is normally used in polarography (5 mV./sec.).

A rotating mercury drop electrode as described by Barendrecht[3] might be used for this purpose. Delahay *et al.*[4] mentioned the use of a similar electrode for anodic stripping, but they did not give experimental results. The platinum electrodes of Barendrecht and Delahay were gold-plated; according to Kemula *et al.*[5], gold interferes during stripping of zinc and cadmium amalgams.

We carried out experiments with an electrode consisting of a platinum wire of 0·25 mm. diameter sealed into the end of a piece of soft glass tubing 5 mm. in diameter and filed flush with the glass as described by Ross *et al.*[6]. The glass tubing was fused to a cylinder of a chromium-iron alloy connected to the shaft of a synchronous motor (3,000 r.p.m.). Electrical contact was made via a graphite sliding contact. The platinum electrode was centred to better than 25μ.

The platinum was cleaned by the method of Ross *et al.*[6] and a mercury drop was made by constant-current electrolysis in a concentrated solution of mercurous nitrate, 1 N in nitric acid, using a mercury pool anode (2 m.amp. 3 min.). During rotation of the electrode, the mercury drop remains attached to the platinum.

For trace analysis an anodic stripping voltammogram is run after a period of cathodic electrolysis of a few minutes. In Fig. 1 are shown the anodic current peaks of zinc, cadmium, lead and copper in a 10^{-3} M potassium chloride supporting electrolyte, 2×10^{-6} M in zinc, cadmium, lead and copper ions. The solution was electrolysed for 5 min. at −1·5 V., the rate of scan during stripping was 5 mV./sec. and silver wire coated with silver chloride served as a reference electrode.

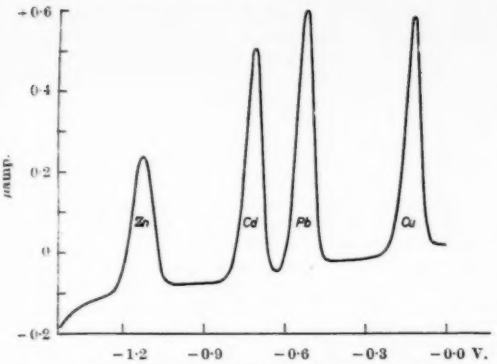

Fig. 1. Anodic current peaks of zinc, cadmium, lead and copper in 10^{-3} *M* potassium chloride, 2×10^{-6} *M* in the respective ions. Cathodic electrolysis: 5 min. at −1·5 V. vs. silver/silver chloride electrode. Rate of scan: 5 mV./sec.

The peaks are well separated and the areas of the cadmium, lead and copper peaks correspond to approximately 7×10^{-6} coulombs. The abnormal area and shape of the zinc peak may be attributed to the influence of platinum on dissolution of zinc from an amalgam, as mentioned by Kemula *et al.*[7].

The time-constant of about 4 sec. for the dissolution process allows the use of the rotating mercury drop for trace analysis of metal ions, described by van Swaay and Deelder (see following communication) as coulometric polarography. We intend to publish a mathematical treatment of this dissolution process in the near future.

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[3] Barendrecht, E., *Nature*, **181**, 764 (1958).
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Trace Analysis with a Rotating Hanging Mercury Drop

By Dr. M. van SWAAY and R. S. DEELDER

Laboratory of Instrumental Analysis, Technical University, Eindhoven

Kemula[1] and Engelsman (preceding communication) have described a technique for the analysis of trace metallic impurities. A solution containing the trace impurities as metal ions is electrolysed under polarographic conditions with a rotating mercury drop cathode. With the resulting drop of dilute amalgam, an anodic polarogram is recorded whereby the metals, dissolved in the mercury, return to the solution. The resulting anodic current peaks correspond to the composition of the amalgam, which, in turn, is determined by the cathodic electrolysis time and the composition of the sample electrolyte.

We should like to describe the technique as 'coulometric polarography'. A refinement of this technique will be described, together with some results showing the applicability of the method to the analysis of non-amalgamating metals.

Both Engelsman and Kemula use the same electrolyte for the cathodic and anodic electrolyses. If different electrolytes are used for the two parts of the cycle, we are free to choose one or more 'stripping' electrolytes for optimum separation of the anodic current peaks. Moreover, the properties of the sample solution are less critical, so that time of preparation may often be reduced. The results depicted in Fig. 2 demonstrate this feature. Here traces of zinc, copper, nickel and chromium in a sodium phosphate sample solution were determined without noticeable interference from the phosphate medium.

Merck analytical grade reagents were used throughout. Water was purified in a mixed-bed ion-exchange column. Supporting electrolytes were purified by prolonged electrolysis with a mercury pool cathode and stored under nitrogen in glass vessels connected to the electrolysis cell via 'Teflon'-glass stopcocks.

The rotating electrode consisted of a 0·5 mm. platinum wire forced through a 0·45 mm. hole in a small 'Teflon' plug contained in the 1½-mm. bore of a short length of glass capillary tubing. The complete electrode assembly was ground flush with fine emery paper. The capillary was mounted with wax on the shaft of a 3,000 r.p.m. synchronous motor running on ball bearings. Centring to within 5μ was achieved by trial and error with the help of a binocular microscope and a stroboscopic flash unit. Electrical contact was made via a small insulated mercury well at the top of the motor shaft.

At the electrode, a mercury drop was generated by electrolysis in a saturated solution of mercurous nitrate. The size of the drop was deduced from the time-integral of the generating current. A single drop could be used for a large number of experiments. By the judicious use of stopcocks, a complete set of experiments could be made without opening the system to the atmosphere.

In Fig. 1 a schematic diagram is given for a typical electrolysis programme. In Fig. 2 a typical anodic

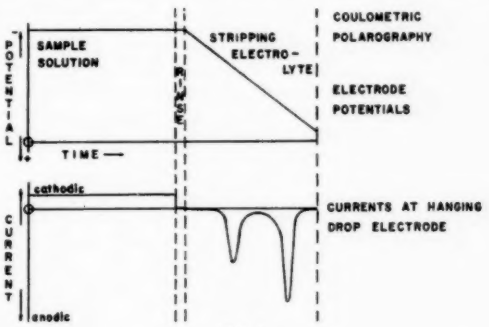

Fig. 1

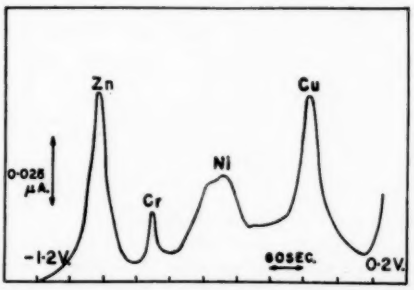

Fig. 2. Sample solution: 1 *M* Na_2HPO_4 with 5×10^{-7} *M* each of zinc, chromium, nickel, cupric ions. Electrolysis, 300 sec. at −1·2 V. (saturated calomel electrodes). Stripping solution: 0·1 *M* KCNS. Voltage scan, −1·2 V. to −0·2 V. at 2 mV./sec.

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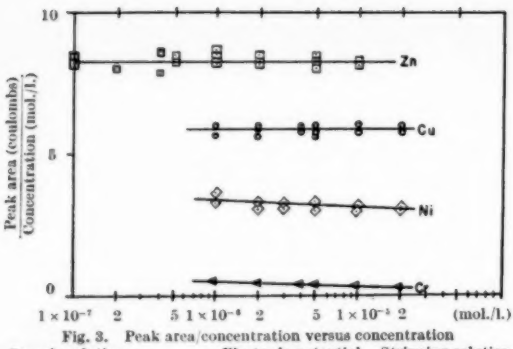

Fig. 3. Peak area/concentration versus concentration

Sample solution	Electrode potential	Stripping solution
Cu^{2+} in 0·1 *M* KCNS	− 1·0 V. (S.C.E.)	0·1 *M* KCNS
Zn^{2+} in 1 *M* KCL	− 1·2 V.	1 *M* KCL
Ni^{2+} in 1 *M* NH_3/NH_4Cl	− 1·35 V.	1 *M* NH_3/NH_4Cl
Cr^{3+} in 0·1 *M* KCNS	− 1·5 V.	0·1 *M* KCNS

Cathodic electrolysis time, 300 sec.
Anodic voltage scan, 2 mV./sec. } for all runs

voltammogram is shown for an analysis of 5×10^{-7} *M* each of zinc, cupric, chromium and nickel ions. In Fig. 3 the relation between peak area and sample composition is shown for the same four metals. The electrolysis programme is described in the legend.

The graphs for nickel and chromium ions show a slight deviation from linearity, which necessitates the use of a calibration curve. The anodic current peaks for these metals also show a tendency to broaden out rather than increase in height with increasing concentration. However, these effects do not seriously impair the usefulness of the method for these 'non-amalgamating' metals.

Attempts to extend the voltage-range beyond −1·5 V. (saturated calomel electrode) have so far been unsuccessful, because a large irreversible cathodic current began to flow at this potential. The nature of the corresponding electrode process is not quite clear, but two possibilities suggest themselves :

(1) The protecting nitrogen gas may not have been sufficiently free of oxygen to prevent reduction of oxygen to hydroxyl or peroxide, which may be responsible for the reduction current. It is unlikely, however, that this process accounts for all the current.

(2) The mercury drop may not have covered the platinum tip completely, so that reduction of hydrogen ions could take place at the exposed part of the platinum. This effect may be expected to show a strong dependence on the *p*H of the solution.

We intend to investigate both possibilities further in this Laboratory. If it proves possible to eliminate these interfering currents, the method will become useful for the analysis of a large group of metals in concentrations down to 10^{-9} *M* and possibly less.

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FLUID–FLUID INTERFACES IN STEADY MOTION

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GIVEN a capillary channel of constant cross-section (shape, area and orientation) and let a fluid, for example a wetting liquid, displace another immiscible one therefrom at a constant rate. The fluid–fluid interface, therefore, will be advancing at a steady velocity. I now set out to prove that this interface will everywhere and continuously have constant curvature, neglecting, as a simplification, distortions due to gravity.

The question is an important one, touched on briefly in the subject-matter and cited literature of the papers by Scriven *et al.*[1,2], and it is an essential one to the discussion of capillary flow if valid boundary conditions are to be assigned in solving the appropriate equations of motion[3].

By simple definition, a constant curvature surface (in the present case, a fluid–fluid interface having a closed-perimeter contact with the pore wall) is one where the sum of the reciprocals of the principal radii of curvature has the same value at every point on the surface. Elementary considerations lead to the realization that the curvature of a fluid–fluid interface at a particular point is proportional to the pressure difference between fluid pressures in each phase at contiguous points across the interface (that is, the so-called capillary pressure)[4].

Without loss of generality, let us limit attention to displacement phenomena in circular capillary tubes ; therefore, the interface either will correspond to the surface of a section of a sphere, if it is a constant curvature surface (as I believe), or the interface will have some other irregular shape (albeit with symmetry to the axis of the tube). In any event, since the motion is given as steady-state, the geometry of the interface will be fixed in time, without reference to the question whether or not a film (that is, a high concentration of surfactants) contaminates the interfacial region.

Suppose we think of a horizontal tube, and of a wetting liquid spontaneously displacing another less-wetting liquid of the same viscosity (assuring a steady motion) and density (avoiding all gravity constraints). The tube will be taken as very long, so that for most of the time when interface motion is occurring the losses of energy where the tube-ends are connected to inflow and outflow reservoirs can be neglected.

An observation of importance is that the axial component of fluid particle velocity will form (approximately) a parabolic profile in the radial direction at all distant positions ahead of and behind the advancing interface, whereas the interface itself moves as though the radial profile of the axial velocity component were (approximately) planar. The earliest references to this consideration which have come to my attention[5,6] are those cited by Gardescu[7].

As a term having phenomenological merit, we introduce the designation, 'fountain effect', to describe how the wetting liquid particles from the central pore space decelerate as they approach the slower moving interface region, acquiring a finite radial component of velocity as they spill over towards the

wall region being vacated by the advancing interface. Correspondingly, a 'reverse fountain effect' is seen in the non-wetting fluid ahead of the interface, as fluid is displaced from the wall region and for the most part moves towards the tube axis, and as the fluid particles accelerate centrally ahead of the advancing interface.

Apparently, an integration of the equations of Navier and Stokes would yield a complete description of the fountain effect, and even though the boundary conditions may be specified, the integrability is hampered by the inherent non-linearities of the problem. Relevant numerical approximations have not as yet been attempted, as can be seen in the literature we have examined. In any event, the speculation of Barrer[8], as investigated experimentally in this laboratory at present[9], that the curvature of a moving interface depends on rate, undoubtedly is an important consequence of the fountain effect. That is, the changing interfacial curvature as rate of interface motion changes means that the capillary driving force is diminished effectively as an external stress is imposed to make the imbibing interface travel faster (that is, the rule of Le Chatelier).

Proceeding from Barrer's point of view, one might argue that the fast-moving fluid particles approaching the slower-moving interface along the central stream gain pressure energy in the loss of kinetic energy; hence, the interface is flattened, and in an extreme case the interface may even reverse curvature. This argument is incomplete, however, in simply emphasizing the Bernoulli condition without reference to viscous dissipation of energy; moreover, by itself no conclusion can be formed from the argument whether or not the moving interface has constant curvature throughout its areal extent.

Of interest in this connexion is the early work of Prandtl *et al.*[10], as extended later by Schlichting[11], who treated the somewhat corresponding problem of the transition in velocity distribution in the region near the entrance of a pipe. The boundary-layer methods, which are applied, have only limited application to the analysis of the fountain effect problem; however, some useful ideas are suggested by this cited work.

For example, taking the Lagrangian point of view (where the moving interface is thought to have a fixed position in the sense that the observer is moving with the same velocity as the interface), the wetting fluid will seem to have an outward component of radial velocity behind the advancing interface, and the non-wetting fluid will seem to have an inward component of radial velocity ahead of the advancing interface. Therefore, at all points on the surface of the interface the tangential component of velocity must be zero. This is because[12], if there is a two-dimensional solid film at the interface, the fluid particles of the two phases on each side must have the same tangential velocity as the film; likewise, if there is no film, the viscous stress will be continuous. Therefore, in either case, no tangential motion can occur in the interface region.

The foregoing continuity proposition is so important that it bears restatement: if all fluid particles in the near-vicinity of one side of the interface have positive components of radial velocity, while those of the second phase in the near-vicinity of the other side have negative components, and if the fluid particles of the two phases making up the interfacial region have the same tangential component of velocity, then this tangential component necessarily is zero at all points defining the interfacial surface.

Now, it will be readily apparent that, if the tangential component of the velocity vector is zero at the boundary of each phase forming the interfacial region, no pressure gradients exist in either phase tangential to the surface. In consequence, the capillary pressure differences between wetting and non-wetting fluids at each point in the interface region will have some constant value; hence the moving interface will be constant curvature throughout. This proposition, once accepted, leads to an obvious simplification in stating the boundary conditions necessary to analyse certain categories of capillary flows (Fara, H., and Rose, W., unpublished).

By parallel argument, we may conclude that interfaces in steady motion will be characterized by constant curvature no matter what the pore shape is, for tangential motions in the interface (whether it be a two-dimensional solid or liquid film) cannot occur. The criteria of Boussinesq[1], namely, the surface shear viscosity and the dilational surface viscosity, have no particular bearing on the problem at hand, but only enter into the description of the dynamics of interface movement during unsteady-states and/or when the cross-section of the pore channel changes with distance[13].

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INTRACELLULAR DISTRIBUTION PATTERN OF RAT LIVER GLUTAMIC-OXALACETIC TRANSAMINASE

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In recent years, serum glutamic-oxalacetic transaminase (GOT)-levels have been studied in many diseases in human beings and a variety of experimental states in animals[1]. Since it is possible that the increased serum GOT activity observed under abnormal conditions is a reflexion of tissue injury (hepatic, cardiac, etc.) resulting in the release of intracellular GOT which then enters the circulation, information concerning the intracellular localization of the enzyme is of basic interest. However, at the time the work

described here was initiated (1958), detailed data were lacking on this point. Hird and Rowsell[2] had reported that rat liver mitochondria and supernatant fluid catalysed transamination between glutamate and aspartate. Müller and Leuthardt[3] found 32 per cent of the total homogenate GOT activity in the mitochondria isolated from isotonic potassium chloride homogenates of rat liver, but the enzyme was concentrated 2·3-fold in this fraction with respect to the original homogenate; the remainder of the activity was apparently present in an unseparated microsomal-supernatant fluid fraction.

In view of the incompleteness of these results, a study was undertaken in our laboratory[4] of the distribution of this enzyme among sub-cellular fractions obtained from rat liver and spleen homogenates by differential centrifugation. GOT activity was assayed by two procedures; that used most frequently was a modification of the spectrophotometric method of Karmen[5] developed for serum, and the other was the method of Greenwood and Greenbaum[6]. All tests were carried out at *p*H 7·6 in 0·033 *M* potassium phosphate buffer.

Normal male Wistar rats (200–400 gm.), fed a stock diet of 'Purina' fox chow checkers *ad libitum* to the time of death, were used. The animals were anæsthetized lightly with ether, bled by heart puncture, and the livers quickly excised and washed free of blood. Usually, 1–3 gm. of liver were removed from the left lateral lobe and homogenized with 9 vol. of ice-cold 0·25 *M* sucrose in a TenBroeck glass grinder. Homogenates were fractionated according to the method of Schneider and Hogeboom[7], with some changes[8], and nitrogen analyses on homogenates and all fractions were made by a micro-Kjeldahl procedure. About 50–70 per cent (average, 62 per cent) of the total GOT activity was found in the mitochondria, 20–40 per cent (average, 29 per cent) in the supernatant (soluble) fraction, and the remainder, due to mitochondrial contamination, in the nuclei (2 per cent) and microsomes (7 per cent). Only the mitochondrial enzyme had a higher specific activity on a nitrogen basis than that of the original homogenate (2 : 1), the specific activity of the supernatant being about equal to the homogenate activity. These results raised the following question: Is part of the enzyme associated with the soluble phase of the intact liver cell or does its recovery in this fraction represent an artefact caused by the procedure employed?

We carried out several series of experiments[4] aimed at shedding further light on this problem. First, it was found that the unusual bimodal distribution pattern of GOT was unchanged when the fractionation medium was varied to favour the preservation of mitochondrial structure or for other reasons. Second, attempts were made to release GOT from the mitochondria by various physical and chemical treatments. Although exposure of the mitochondria (or homogenate) to the action of a Waring blendor, sonic oscillation, distilled water, or the detergent, 'Triton *X*-100', unmasked previously unreactive enzyme, that is, increased the measured GOT activity to varying degrees but several-fold in general, only a portion, at best, of the total mitochondrial activity was solubilized in the process. It should be emphasized that since the total activity of GOT of freshly isolated, intact mitochondria suspended in sucrose is at least doubled by such treatments, the activity of GOT recovered in the supernatant fraction is actually much less than 30 per cent of the total GOT present in the whole cell.

On the basis of these experiments and the specific activity data mentioned previously, it was concluded[4] that GOT is concentrated in the mitochondria of the normal rat liver cell. The picture was more equivocal concerning the activity found in the supernatant fluid. However, the fact that a significant amount of the enzyme was always present in this fraction, regardless of the nature of the homogenization and fractionation medium, spoke for the localization of some GOT in this part of the intact cell, and posed the question of whether there were any differences in the properties of the enzyme recovered in the mitochondria and supernatant. One step in examining this question is to investigate the activities of the enzyme in response to certain physiological stresses. In preliminary experiments, we studied the effects of starvation (72 hr.) and whole-body X-irradiation (700 r.) on the specific activities of GOT in mitochondrial and supernatant fractions of rat liver. While there was some indication that the activities of the two fractions responded differently, particularly after irradiation, the small number of animals involved precluded the drawing of definite conclusions. Interesting observations bearing on this question have been reported recently by Fleisher *et al.*[9] and Rosenthal *et al.*[10]. By a paper electrophoretic technique, the former authors demonstrated two fractions of GOT activity in water extracts of human and dog heart and liver minced in a blendor. One fraction migrated towards the anode and showed the known characteristics of serum GOT, while the other migrated towards the cathode and exhibited different affinities for α-ketoglutarate and L-aspartate and a different *p*H-activity curve. In our work[4], an attempt had been made only to differentiate the readily measurable mitochondrial GOT from that part of the mitochondrial activity which was unmasked and solubilized on blending, by assaying GOT at various *p*H values between 5·4 and 8. No difference was observed. Although the effect of *p*H on the activities of the supernatant and mitochondrial enzymes was not investigated, we entertained the possibility that the bifractional distribution of GOT might be related to the observation of Fleisher *et al.*[9]. Experimental support for such a relationship appeared in the recent abstract of Rosenthal *et al.*[10]. In agreement with our findings, although a completely different enzyme assay was used, they reported that GOT was found in the mitochondrial (70–80 per cent) and soluble fractions prepared from 0·25 *M* sucrose homogenates of rat liver. Furthermore, they indicated that the mitochondrial and soluble GOT activities of rat liver closely resembled the cationic and anionic forms of the enzyme described by Fleisher *et al.*[9] with respect to *p*H-activity curves and relative affinities for aspartate and α-ketoglutarate.

Regarding the intracellular localization of the enzyme, May *et al.*[11] recently found 45 per cent of the total GOT activity of rat liver homogenate in the mitochondria, 17 per cent in the supernatant fluid and 33 per cent in the nuclear fraction. An appreciable amount of GOT was also recovered in the soluble fraction prepared from rat brain homogenate[11]. We have shown that while GOT is apparently concentrated in the mitochondria of rat spleen[12], considerable transaminase activity is present in the supernatant fraction obtained from 0·25 *M* sucrose homogenates of this organ. It would seem that a bimodal distribution pattern of GOT is characteristic of many tissues, and it would be of interest to determ-

ine if the enzyme also exhibits chemical differences in the two fractions from tissues other than liver. In this connexion, it should be noted that the cationic and anionic forms of GOT were found in both heart and liver[9].

Fleisher *et al.*[9] showed that serum from several patients with myocardial infarction and viral hepatitis contained only small amounts of cationic GOT. From this, it might be inferred that in these diseases increases in serum GOT result chiefly from the release of the soluble, anionic intracellular GOT rather than the particle-bound, cationic enzyme. This would be consistent with the observation already made that, at least in rat liver, mitochondrial GOT is released in soluble form only after considerable structural damage to the particles. In this respect it is to be noted that the cationic enzyme was demonstrated in soluble preparations made by treating tissues in a blendor[9]. We have found that treatment of rat liver mitochondria (0·25 *M* sucrose) for increasing periods, either in a Waring blendor (2–10 min.) or sonic oscillator (5–20 min.; Raytheon model *DF* 101, 10 kc.), followed by centrifugation of the mitochondria for 1 hr. at 150,000*g*, renders soluble increasing amounts of the total mitochondrial activity present after treatment. However, from our experiments it would appear that about half the enzyme remains firmly bound to the mitochondrial membranes, and possibly the cristæ, and to attempt to release more, at least without special care, might invite denaturation of the GOT. Hence caution should be exercised in assessing and assigning significance to the proportions of the two forms of GOT present in extracts of blended organs[9].

Finally, it may be necessary to re-evaluate past studies in which liver homogenate GOT activity was examined in animals subjected to a variety of experimental conditions, that is, administration of hormones, instillation of toxins, exposure to ionizing radiations, effect of dietary deficiencies, carcinogens, etc. For example, since the soluble enzyme represents about 30 per cent or less of the total homogenate GOT of fresh preparations in sucrose, it can be calculated that as much as a 75 per cent decrease in its specific (or total) activity would yield only a 23 per cent decrease in the specific (or total) activity of overall homogenate GOT, provided there are no changes in the nitrogen content.

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PHARMACOLOGICALLY ACTIVE SUBSTANCES PRESENT IN THE CENTRAL NERVOUS SYSTEM

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PSYCHOPHARMACOLOGY is a new interdisciplinary science. It attempts to correlate mental aberration, emotional disturbance, and behavioural changes with modifications in the normal biochemistry of the central nervous system.

In an attempt to interrelate brain function with the psyche (mind) a number of basic propositions must be acquiesced. Thus, the thesis of psychopharmacology rests upon the concept that the brain and psyche, although distinct entities, are actually intimately associated and mutually dependent. It adheres to the principle that animal behaviour is a consequence of a dynamic equilibrium between a primitive, emotional and instinctual system (rhinencephalon), and a recollective, rational and cognitive mechanism (cerebrum). Psychopharmacology also asserts that emotion may very well be a result of cortical disorganization, and that biochemically induced changes in the central nervous system, via drug action, will modify psychic reactions.

This concept of mind–brain interdependence is not novel. In the sixth century B.C., the Greek physician Alcmaeon taught that the brain was the organ of the mind. To-day, the mind is, in certain respects, still considered to be the subjective expression of the living brain. Certain psychotogenic (hallucinogenic) drugs, for example lysergic acid diethylamide (LSD), mescaline, etc., provide an opportunity to readily induce a reversible model psychosis which, at least superficially, mimics schizophrenia, thus furnishing a closer introspection into possible psychopathological mechanisms.

A host of phrenotropic agents has been introduced into the armamentarium of the psychiatrist and physician in the past several years. These medicaments are believed to act, in the main, by way of certain substances found in the central nervous system. The substances thus far posited to be concerned with brain and mental activities include: adrenaline, noradrenaline, dopamine (3-hydroxytyramine), 5-hydroxytryptamine (5-HT), histamine, acetylcholine, γ-aminobutyric acid (GABA), adrenochrome, and substance '*P*'. Structural configurations for these chemical materials are depicted in Fig. 1.

Also intimately concerned are the sundry enzymes which synthesize and biotransform these biogenic substances. It appears that the neuro-hormones following synthesis are stored, physiologically inert, in certain cytoplasmic organelles and protected by certain intracellular barriers from enzymic catabolism. Following release, these substances become physiologically active, react with some cellular or

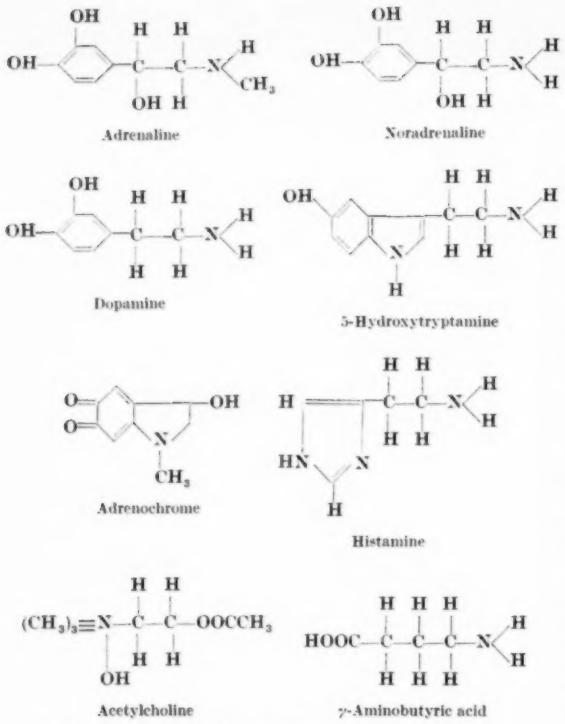

Fig. 1. Graphic configurations of some centrally active compounds

plasmatic membrane receptor, and thence regulate neuronal transmission or other functional processes. Biotransformation of the neuro-hormone then occurs via a specific enzyme system with subsequent termination of the substance's excitatory or inhibitory effects.

The central distribution of the neuro-hormones is well documented. The brain distribution of noradrenaline, the precursor of adrenaline, is disproportionate; highest concentrations are found in 'primitive' brain sites, namely, the hypothalamus, area postrema, medullary reticular formation and medial thalamus. Adrenaline, in lesser amounts, follows this distribution rather closely. Dopamine is located in highest concentrations in the corpus striatum, where peculiarly its metabolite noradrenaline is found only in minor quantities. Conversely, brain loci, for example the hypothalamus, replete in noradrenaline and its biocatalyst, dopamine-β-oxidase, contain paucid quantities of the parent molecule. 5-HT, synthesized from 5-hydroxytryptophan by the agency of aromatic L-amino-acid decarboxylase, follows the localization of noradrenaline rather closely. It is significant that only 1 per cent of the 5-HT content of vertebrates is found in the brain. The area postrema contains relatively high levels of 5-HT, but strangely enough no enzyme for its synthesis has been discovered here. Peripherally ubiquitous histamine is found in significant amounts solely in the hypothalamus and cerebellum, but histaminergic synapses have not been identified. Acetylcholine, reported by Pfeiffer *et al.*[1] to be derived from the naturally prevailing amino-alcohol dimethylaminoethanol, has been discovered particularly in the cerebral cortex, pons, thalamus, basal ganglia and other brain nuclei. An interrelationship between acetylcholine metabolism and that of adrenaline may exist for, theoretically at least, the monomethylaminoethanol chain cleaved from the latter compound may serve as a basis for choline synthesis[2]. GABA, a component of Florey's factor 1, and manufactured via the agency of *l*-glutamic acid decarboxylase on glutamic acid, is present predominantly in the rhinencephalon ('visceral brain'), medulla, pons, and cortex. Adrenochrome, the highly labile indole oxidation product of adrenaline, has been discovered in erythrocytes and heart, and following convulsions, in brain tissue[3]. Substance '*P*', a biologically active polypeptide, has been detected in the intestines and brain, and in the latter especially in the basal ganglia, thalamus, hypothalamus, area postrema, and spinal cord.

A number of functions have been ascribed to these centrally active substances and the *modus operandi* of the various phrenotropic medicaments is largely predicated on their ability to modify the metabolism or receptor binding sites of these hormones or their metabolites. Hess[4] has postulated the existence of a subcortical (hypothalamic) integrating system composed of two reciprocally antagonistic divisions, namely, the 'ergotropic' and the 'trophotropic' nervous systems. The former mediates arousal, and an alerted mental state, skeletal muscle hypertonia, and an exaggeration of sympathetic activity. The latter centre, and anatomically the more anteriorly disposed, is concerned with sleep, apathy and psychic depression, a diminished skeletal muscle tone, and increased parasympathetic activity. Brodie and Shore[5] theorize that free (active) 5-HT and noradrenaline act as synaptic mediators for the trophotropic and ergotropic nervous systems repectively.

Marrazzi[6] has shown that adrenaline, noradrenaline, and 5-HT, as opposed to adrenochrome, are potent intercortical synaptic inhibitors, and he postulates that certain mental disturbances result from an imbalance between inhibition by adrenaline, noradrenaline or 5-HT and facilitation by acetylcholine of central synaptic, cholinergic transmission. Although acetylcholine activates Renshaw cells in the spinal cord, its, noradrenaline's, and 5-HT's inertness following electrophoretic application to medullary and reticular formation neurons implies a non-transmitting role for these substances in these particular areas[7].

Woolley and Shaw[8] have suggested that certain mental disturbances are caused by a metabolic deficiency of brain 5-HT. They have described a rather novel *in vitro* excitant action of this neurohormone on cortical oligodendroglia, and they hypothesize that 5-HT serves to augment cerebral metabolism through this neurotonic mechanism. Unfortunately, the careful clinical results of Feldstein *et al.*[9] do not support the concept of a relationship between 5-HT metabolism and acute or chronic schizophrenia.

The physiological significance of centrally partitioned histamine is still obscure. Indirect evidence for its participation in mental activity is exemplified by the antihistaminics, a number of which possess potent central actions. More direct evidence is gleaned from reports that histamine (and its methylated derivatives) are amine oxidase inhibitors.

Further, certain monoamine oxidase inhibitors affect histaminase (diamine oxidase) activity irreversibly. Histamine appears to be a natural synaptic inhibitor similar to 5-HT. Adrenaline blocks *l*-histidine decarboxylase[10], the enzyme responsible for the biosynthesis of histamine, and it may be that this hormonal effect provides a negative servo-mechanism for the regulation of central histamine-levels.

Feldberg[11] relegates a synaptic transmitting (facilitating) role to acetylcholine in the autonomic and central nervous systems. Because of this action on brain stem reticular formation synapses, Himwich and Rinaldi[12] propose that elevated brain concentrations of acetylcholine may exert antischizophrenic actions.

GABA, an amino-acid metabolite, is a synaptic and neuronal inhibitor. Purpura *et al.*[13] feel that this material is a transmitter substance in the sense that it acts as an inactivator of synaptic depolarization. It has been suggested that it may well be involved in neuro-excitability and in the etiology of certain brain abnormalities, for example epilepsy. GABA may also subserve the action of certain convulsive drugs, and the observation that monoamine oxidase inhibition elevates free brain GABA-levels tempts one to speculate that this explains the mechanism of the anticonvulsant action of certain 'psychic energizers'.

Hoffer[3] postulates that adrenochrome (chemically similar to LSD), or dihydroxyindole catabolites of abnormal adrenaline oxidation, are causes of schizophrenia. This concept, however, has been considerably criticized and this worker's findings have not been consistently substantiated. Adrenochrome may still have significance, nevertheless, for this hallucinogen is, at least *in vitro*, an inhibitor of both glutamic acid decarboxylase and cholinesterase.

Substance '*P*' is given a neuro-active role in the dorsal horns of the spinal axis, and a non-cholinergic transmitting function in the autonomic nervous system. It has been likened to a type of 'physiological tranquillizer' because of its meprobamate-like polysynaptic inhibitory properties in the central nervous system.

Vitally concerned with the action of the central neuro-hormones, especially the biogenic catecholamines and 5-HT, are the enzymes responsible for their biotransformation and inactivation. Monoamine oxidase is a prime example of such a biocatalyst the activity of which can be modified or destroyed by drug action with either a consequential deviation in normal metabolic pathways followed by its substrates or by a pile-up of these compounds. Monoamine oxidase is widely distributed in the animal body and is primarily located in mitochondria. Centrally, the enzyme is normally found where 'true' cholinesterase-levels are low, namely, the lenticular nucleus, hypothalamus, and pyramidal tracts. The primary functions assigned to monoamine oxidase are the metabolism of the relatively inert metanephrines[14,15] and other O-methylated catecholamine metabolites, and of degradating 5-HT to 5-hydroxyindoleacetic acid[16]. Dopamine, formed from dihydroxyphenylalanine by the action of aromatic L-amino-acid decarboxylase, is a better substrate for monoamine oxidase than noradrenaline, and hence enzyme inhibition by administration of a 'psychic energizer' shunts the metabolic pathway in favour of one catalysed by catechol-O-methyltransferase with the consequential accumulation of 3-methoxytyramine. This latter enzyme has been recently given the principal task of converting noradrenaline and adrenaline to the respective metanephrines[17,18], monoamine oxidase notwithstanding. Spector *et al.*[19] suggest that the latter enzyme may control cellular 5-HT and noradrenaline-levels, while catechol-O-methyltransferase inactivates blood-borne catecholamines. However, Belleau *et al.*[20] claim, rather convincingly, that noradrenaline is not acted on by monoamine oxidase at adrenergic effector cell sites. As may be noted, the implication of aromatic L-amino-acid decarboxylase in the formation of both the catecholamines and 5-HT leads one to believe that a closer correlation exists between the metabolic disposition of the catecholamines and 5-HT than heretofore suspected.

In summary, a number of pharmacologically active substances naturally occurring in the central nervous system have been briefly characterized and their potential physiological functions described. It seems, in retrospect, rather significant that of all the substances postulated to officiate as neuro-mediators or incriminated in the etiology of mental illness, only acetylcholine possesses neuronal and synaptic facilitating properties. It would appear that acetylcholine is the driving force in the nervous system whereas the remaining substances simulate physiological brakes. The phrenotropic agents widely employed in the pharmacotherapy of various forms of mental illness obviously modify brain biochemistry, some perhaps via mitochondrial receptor attachment[21], with apparently consequential effects on the psyche. However, it would be rather premature and naïve to hope that contemporary physiological, biochemical, and even psychopharmacological techniques of studying and modulating brain function will instigate dramatic and important insights into psychic activities. This problem is knotty and may perhaps have no solution as presently assailed. Nevertheless, break-throughs into this twilight zone bridging mind and brain may be possible if studies are performed with determination and if moderately permeated with imagination.

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XUM

ANALYSIS OF THE THYMUS INFLUENCE IN LEUKÆMOGENESIS

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THYMECTOMY markedly reduces the incidence of lymphocytic leukæmia in mice which would otherwise develop the disease spontaneously[1], or following exposure to ionizing radiations[2], chemical carcinogens[3] or virus inoculation[4]. Grafting thymus tissue to thymectomized mice restores the susceptibility to the development of lymphomas[1,3,5,6]. Thymectomy might prevent leukæmogenesis by effecting removal of one or more of the following: (*a*) the source of a leukæmogenic virus (either present from birth or activated by external leukæmogens); (*b*) the site of multiplication of such a virus (assuming that multiplication is a prerequisite for leukæmia induction); (*c*) the only type of cells capable of undergoing leukæmic transformation; (*d*) the source of a humoral factor involved in leukæmogenesis.

Presence of Leukæmogenic Virus in Non-Thymic Tissues

Evidence has been presented in previous papers[6,7] to show that the thymus does not act by simply providing the source of a leukæmogenic virus. Recovery of the agent from non-leukæmic tissues of virus-infected thymectomized mice[7] and restoration of incidence of lymphoma in such mice by grafting thymuses from normal mice as late as six months after thymectomy[6] strongly suggest that the virus is widely distributed in its host and not simply confined to the thymus. These results have recently been confirmed by other workers[8].

Virus Multiplication in Thymectomized Mice

It is possible that a leukæmogenic virus must reach a critical concentration before it can induce leukæmia in its host and that multiplication can only occur in thymus tissue. The question whether virus can multiply outside thymus tissue is being studied in this laboratory. Extracts of non-leukæmic tissues of thymectomized, virus-inoculated mice have been injected into successive generations of thymectomized mice after the operation. Since it has been shown that new-born or suckling mice are more susceptible to leukæmic filtrates than adult mice[9] it was decided to perform the operation of thymectomy in the first week of life. The technique used for baby mice was similar to that employed in this laboratory for adult mice[6], and the immediate operative mortality was no higher than 15 per cent. Subsequent mortality, however, was very high in mice that had been thymectomized at 1 day of age, more than 50 per cent of these dying between 2 and 4 months (whether they had been inoculated with virus or not), suggesting that the thymus at birth may be essential to life. Mortality at 3 months of age was much lower in mice thymectomized between 3 and 5 days of age. A group of mice thymectomized at that age were inoculated with leukæmic virus one day after the operation and killed 2–3 months later in order to prepare extracts from their spleens, livers and brains. Checks were made for the total absence of thymus tissue at death. The extracts were inoculated into a group of intact 3–5-day-old mice and into a second group of 3–5-day-old thymectomized mice. Extracts were prepared from the latter when they were about 3 months old and were inoculated in turn into further groups of intact and thymectomized baby mice. The incidence of lymphomas in the intact mice receiving the extracts at each generation is recorded in Table 1.

Table 1. Leukæmogenesis in Intact C3*Hf/Gs* Mice by Virus Serially Passaged through Thymectomized Mice

Inoculation given	No. of intact mice treated	Leukæmia incidence (per cent) and age in months
Passage *A* filtrate	26	73 (3–6)
Virus passaged once through thymectomized mice	10	80 (3–6)
Virus passaged twice through thymectomized mice	8	63 (4–6)
Virus passaged thrice through thymectomized mice	11	73 (3–7)

Only preliminary results from three generations are available, and these suggest that the leukæmogenic virus has not been lost by serial transfer through thymectomized hosts. The possibility, therefore, remains open that multiplication might occur in extra-thymic tissues. Such extra-thymic multiplication is not accompanied by neoplastic transformation in lymphoid cells, and, as a corollary, it may be that only replication within the thymus can be associated with leukæmogenesis.

Origin of Leukæmic Cells

The next problem to be considered is whether the thymus acts as a source of potentially malignant cells. It is possible that thymus lymphocytes differ from other lymphocytes in their susceptibility to neoplastic transformation. Experiments employing thymectomized hosts either genetically[9,10,11] or immunologically[12] tolerant of foreign thymus grafts have been set up by various workers in an attempt to settle this question. The results depended on the transplantation behaviour of the lymphoid tumour arising from the grafted thymus. In some cases the tumour behaved as if it had originated from lineal descendants of cells regenerating from the donor thymus tissue[11,12], which could then be assumed to have acted as a source of potentially malignant cells. In other cases, however, the neoplastic cells had the immunogenetic character of host-type cells[10,12] which must have populated the graft. Transplantation behaviour of tumour cells that have resided in an F_1 environment is not always clear cut, especially when the parental strains do not differ at the *H*-2 locus, and is always open to criticism in view of the phenomenon described by Barrett and Deringer[13].

The problem of the origin of leukæmic cells has recently been studied here in $(AkXT_6)F_1$ hybrid

mice the cells of which can be identified at metaphase by the presence of an unusually small chromosome. The hybrids were inoculated at birth with leukæmic filtrate, thymectomized at 1 month of age and grafted subcutaneously with an intact thymus from a healthy 1-day-old *Ak* or T_6 mouse. The tumours developing in the graft were transplanted to *Ak*, T_6 and F_1 mice and also analysed cytologically for the presence or absence of the T_6 marker chromosome. Preliminary results are shown in Tables 2 and 3, from which it is apparent that: (*a*) the *Ak* thymus is much more effective than the T_6 thymus in restoring susceptibility to lymphoma development; (*b*) leukæmic cells arise in most cases from lymphoid cells of the host, suggesting that the thymic influence responsible for the leukæmic change is a non-cellular indirect one.

Table 2. Incidence of Lymphoid Tumours in *Ak* or T_6 Thymuses grafted to Virus-infected $(AkXT_6)F_1$ Mice

Treatment given	No. of mice in group	No. of mice with lymphoid tumours and age at diagnosis
Passage *A* at birth	12	11 (2–4 months)
Passage *A* at birth, thymectomy and *Ak* thymus graft at 1 month	7	5 (3–5 months)
Passage *A* at birth, thymectomy and T_6 thymus graft at 1 month	8	1 (4 months)

Table 3. Immunogenetic and Cytological Analysis of Lymphoid Tumours arising in Thymus Grafts in Virus-inoculated Thymectomized $(AkXT_6)F_1$ Hosts

No. of mouse	Thymus graft	Transplantation results*			Cytological analysis		
		Growth in *Ak*	Growth in F_1	Growth in T_6	No. of *Ak* cells found	No. of F_1 cells found	No. of T_6 cells found
1	*Ak*	0/5	4/6	0/4	3	25	0
2	*Ak*	0/4	6/6	0/3	0	31	0
3	*Ak*	0/5	3/5	0/3	1	20	0
4	*Ak*	3/4	3/5	0/4	34	5	0
5	T_6	0/5	6/8	0/8	0	21	3
6	*Ak*	0/6	6/6	0/3	5	36	0

* Numerator, number of takes; denominator, number of animals.

The Question of a Thymus Hormone

Law[10] was the first to suggest that a non-cellular factor from the thymus might be exerting an influence in leukæmia development. In a series of experiments, as yet unconfirmed, Metcalf has given evidence for the existence of a lymphocytosis-stimulating factor from the thymus and suggested that this factor might be the non-cellular influence by which the thymus appears to determine leukæmogenesis[14]. In our laboratory, repeated injections of normal *Ak* thymus extracts have failed to restore the usual leukæmia incidence in thymectomized *Ak* mice. Non-cellular thymic extracts are thus probably not capable of replacing the thymus environment so far as leukæmogenesis is concerned.

The possibility of a humoral circulating factor from thymic transplants is suggested by the results obtained with intrasplenic thymus grafts. In contrast to subcutaneous grafts no lymphoid tumours arose from intrasplenic grafts in thymectomized irradiated mice[15], suggesting that a circulating leukæmic factor had been removed by the liver. In this laboratory, however, thymuses from intact day-old mice transplanted into the spleen of thymectomized virus-inoculated mice of the isogenic line have developed lymphocytic neoplasms, but the incidence of such tumours has been lower than that recorded for subcutaneous grafts in parallel experiments (Table 4).

Table 4. Development of Lymphocytic Neoplasms in Thymectomized C3*Hf*/*Gs* Mice bearing Intrasplenic Thymus Grafts

Inoculation at birth	Site of thymus graft*	No. of mice in group	No. of mice developing lymphoid tumours and age at diagnosis	Incidence (per cent)
Passage *A*	subcutaneous	26	20 (3–8 months)	77
Passage *A*	intrasplenic	38	17 (3–5 months)	45
Nil	intrasplenic	14	0	0

* Grafts were performed in all cases 6 days after thymectomy at 1 month of age.

Conclusions

It is apparent from the above series of experiments that the thymus does not influence leukæmogenesis by providing the source of a leukæmogenic virus nor the cells most susceptible to leukæmic transformation. It is doubtful whether it is required merely as a growth medium for the virus. It is known, however, that the thymus provides an environment wherein lymphocytes (whether produced in the thymus itself or by the rest of the lymphoid complex) can proliferate much more actively than in any other lymphoid tissue[16]. What determines this high local proliferative activity is unknown: it may be the non-cellular factor mentioned above or Metcalf's lymphocytosis-stimulating factor. It is possible, therefore, that neoplastic change occurs in virus-infected lymphocytes only if they proliferate at a rate characteristic of thymus lymphocytes. This suggestion could be put to experimental test, for example, by preventing virus-infected host lymphocytes from entering the thymus by isolating the latter in the anterior chamber of the eye or in a millipore diffusion chamber. Experiments of this type have been in progress for some time and their results will be published and discussed at a later date.

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EFFECTS OF HEAVY WATER ON POLIOVIRUS MULTIPLICATION: RESULTS AND SPECULATIONS ON MECHANISM

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THE multiplication of poliovirus (*MEF*-1 or *CHAT* strain) in cells previously grown in media containing deuterium oxide results in a five- to ten-fold increase in burst size[1,2]. In these experiments, two stable tissue culture lines (HeLa and *MS*) and freshly explanted cells of rhesus monkey kidney were grown in concentrations of deuterium oxide, ranging between 20 and 40 per cent, prior to exposure to poliovirus. Incubation of these cultures at 37° C. resulted in increased virus multiplication in all the strains tested. When they were incubated at 40° C., virus strains which would not normally replicate at this temperature grew in the cultures. One example was the attenuated type 1 virus, the *CHAT* strain, which did not grow on monkey kidney cells at 40° C. in water medium, but replicated when the growth medium contained deuterium oxide or 50 per cent concentration in the overlay.

More recently, Lwoff and Lwoff[3,4] have described the effects of high concentrations of deuterium oxide on the multiplication of poliovirus I, rt. 38·5° at different temperatures, and have discussed possible mechanisms responsible for the results. In their experiments they added deuterium oxide (50 or 75 per cent) to a stable tissue culture line of human cancer cells, the *KB* line, immediately after infection with virus and found marked differences in virus yield as compared with water media in relation to the incubation temperature of the cultures. Thus at 33° C. there was a marked reduction in virus yield; at 37° C. more virus was produced; and considerably greater multiplication of virus occurred at 39·5°. Lower concentrations of deuterium oxide were apparently not tested, or had little or no effect.

Study of progeny of poliovirus grown in deuterium oxide at 40° C. The mutagenicity of deuterium oxide in certain bacterial and phage systems has been recorded[5–7] and the possibility that growth of poliovirus in deuterium oxide at elevated temperatures may give rise to mutants was investigated. The test system consisted of monkey kidney cell monolayers exposed to the attenuated *CHAT* or the virulent Mahoney strains of type I poliovirus and incubated at 37° and 40° C. respectively. One set of virus cultures was maintained at 37° C. throughout the experiment. A duplicate set was kept at 40° C. for 18 hr., then transferred to an incubator held at 37° C. Four days later the number of plaques appearing in all the plates were counted and ratios of those observed in cultures kept for a limited period at 40° C. to those observed in cultures kept consistently at 37° C. calculated. The results shown in the last column of Table 1 are expressed as differences between plaque ratios obtained for the test virus and standard *CHAT* virus.

The character of *CHAT* virus grown in deuterium oxide at 37° C. has not changed whereas different plaque ratios have been obtained for *CHAT* virus grown in deuterium oxide at 40° C. However, it should be emphasized that these ratios were obtained for virus grown in cultures kept at 40° C. for a limited time only (18 hr.). If cultures were kept at 40° C. during the entire incubation period no plaques of *CHAT* virus–deuterium progeny were detected. Since, however, growth of this progeny in cultures exposed to 40° C. for a limited time has also been observed following second tissue culture passage (without added deuterium oxide) it is felt that its character differs from that of the parental *CHAT* virus.

Table 1. Ability of Virus Strains to reproduce at 40° C.

Virus strain	No. of exps.	No. ratios determined	Ratio 40°/37° plaques*
Mahoney	9	9	+ 1·64 ± 0·36†
37°-D_2O-*CHAT*	7	8	− 0·02 ± 0·03
40°-D_2O-*CHAT*	7	12	+ 0·28 ± 0·03

* Ratio 40°/37° test virus—ratio 40°/37° *CHAT*.
† S.E.

Comparative effect of deuterium oxide and X-rays. Speculations concerning the mechanisms of action of deuterium oxide should take into account the fact[2,8] that tissue culture cells grown in deuterium oxide tend to become enlarged. Virus multiplication is increased in X-ray-produced giant cells[9], and it has been shown that the size of the irradiated cells is due to continued synthesis of large molecules in cells that have lost their ability to divide[10]. In preliminary experiments the *CHAT* strain of type I poliovirus[11] was grown on monkey kidney cell monolayers maintained in (*a*) normal growth medium, (*b*) in deuterium oxide and (*c*) irradiated by 300 r. Duplicate cultures were kept at 35° and 40° C. respectively. As judged by the average plaque size of the virus shown in Table 2, the effect of deuterium oxide seemed to be more pronounced than that of X-rays, but these conclusions may be revised after these cells have been subjected to different dosages of X-rays.

Table 2. Effects of Deuterium Oxide and of X-Irradiation (300 r.) on the Multiplication of *CHAT* Poliovirus on *MK* Cells at 35° C. and 40° C.

Medium	X-ray	D_2O (per cent)		Average plaque size 35° C.	No. of plaques per plate 40° C.
		Medium	Overlay		
H_2O	—	—	—	2·60	0
D_2O	—	40	—	2·63	0
H_2O	+	—	—	3·00	0·5
D_2O	—	—	50	3·14	4·0
D_2O	—	40	50	3·66	9·0

Speculations concerning mechanism of action of deuterium oxide. The mechanism by which deuterium oxide influences virus growth would appear to involve the extent of randomization of the virus structure and could manifest itself either by altering the stability of the hydrogen bonding in the molecule or by affecting the transition temperature of the helix-random coil transformation. Lwoff[12,13] has discussed the effects of temperature on virus multiplication.

Blout and Lenormant[14] have shown that the strong infra-red absorption bands of deoxyribonucleic acid (DNA) observed at 1,680 and 1,645 cm.$^{-1}$ are shifted to 1,660 and 1,625 cm.$^{-1}$ in deuterium oxide solution. The 1,660 and 1,625 bands are also observed with dry DNA and they conclude that this shift indicates stronger hydrogen bonding. A similar shift in the infra-red spectrum of RNA has now also been observed[15,16]. Thus the absorption which RNA normally displays in the 1,700–1,720 cm.$^{-1}$ region is shifted to 1,670–1,690 cm.$^{-1}$ in deuterium oxide solution. This absorption is thought to reflect base interaction (as between adenine and uracil) and to involve proton transfer.

The general effect of deuterium substitution on the strength of hydrogen bonds and on helix random coil transformations has been discussed by Scheraga[17]; and more recently it has been shown that differences in helix-coil transition temperatures depend on the specific chemical system under study being reduced by deuteration in poly-γ-benzyl-L-glutamate[18] and increased by deuteration in ribonuclease[19].

The deuterium effect, however, is most likely dependent on changes in the stability of the hydrogen bonding of the protein and possibly of the RNA. Interaction between the peptide bonds and the aqueous medium could affect the apparent half-life of a given virus particle; and increased stability of the hydrogen bond, such as could be conferred by deuteration, might then lengthen the effective half-life of the virus, resulting in increased multiplication.

Experiments now in progress may shed further light on the mechanism of the action of deuterium oxide.

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MECHANISM OF MUTAGENESIS INDUCED BY FORMALDEHYDE

THE ESSENTIAL ROLE OF THE 6-AMINO GROUP OF ADENYLIC ACID (OR ADENOSINE) IN THE MEDIATION OF THE MUTAGENIC ACTIVITY OF FORMALDEHYDE

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IN a previous communication[1], I have established by means of a chemically defined and axenic treatment medium that formaldehyde exhibits no mutagenic activity towards *Drosophila melanogaster* by the larval feeding method, unless adenylic acid is present in the treatment medium. A reaction-product formed between formaldehyde and adenylic acid in the treatment medium was postulated as the metabolite concerned in the mediation of the mutagenic activity of formaldehyde. The principal metabolite was assumed to be an adenylate dimer ($\overline{AA}$), where two adenylic acid molecules are linked together through their amino-groups by a methylene bridge. The stereochemical properties of this metabolite and its subsequent utilization in deoxyribonucleic acid synthesis, together with mechanisms for its mutagenic activity, were discussed[1].

An essential role was thus ascribed to the 6-amino group of adenylate for the formation of the adenylate dimer, since the binding of formaldehyde by ribonucleic acid mono- or poly-nucleotides appears to be a specific function of the amino-groups of their bases[2]. It is now found that adenylate is effective when present in the treatment medium as any of the forms adenosine-3′-phosphate, adenosine-5′-phosphate, or deoxyadenosine-5′-phosphate. The simple replacement of the 6-amino group of adenosine and adenosine-5′-phosphate by a hydroxyl group (to give inosine and inosine-5′-phosphate) completely removes the ability of these compounds to mediate the mutagenic activity of formaldehyde. Although adenosine is as effective as the adenine ribonucleotides, adenine shows no ability to mediate formaldehyde-induced mutagenesis and is actually anti-mutagenic on a formaldehyde-containing yeast–glucose treatment medium[3]. Adenine should be able to form a metabolite by reaction with formaldehyde similar to the one postulated for adenylate; but its free imino-group at the 9-position of the purine ring may be blocked by additional reaction with formaldehyde[4], thus preventing subsequent ribose sugar attachment; this condition would be expected to debar the utilization of adenine in nucleic acid synthesis.

The design of experiments[1,5], the method of treatment of larvæ[3], and brood pattern determinations of sex-linked recessive lethal mutation-rates[3] are as previously described. However, it has been found that a concentration of formaldehyde of

$1{\cdot}0 \times 10^{-2}$ *M*, as against a previous concentration of $2{\cdot}0 \times 10^{-2}$ *M*, results in an improvement in the mutational response and in the survival and development of the larvæ on the synthetic medium. This improved concentration was therefore used, as it more markedly illustrates the difference of effect of the various purine derivatives on the mutagenic activity of formaldehyde. Each nucleotide or nucleoside is used at a concentration of 0·1 per cent; adenine is used at an approximately equimolar concentration of 0·05 per cent. Ordinary medium is the oatmeal–treacle medium normally used for culturing *Drosophila* in this laboratory.

Table 1. Effect of Adenosine, Adenosine-5′-phosphate, Inosine and Inosine-5′-phosphate on the Mutagenic Activity of Formaldehyde when Newly-hatched Larvae are treated for 72 hr.

Treatment medium	*r*A	*r*H	*r*A5′-P	*r*H5′-P
Molarity of formaldehyde	$1{\cdot}0 \times 10^{-2}$			
Total larval developmental time in days	5·0	5·3	5·0	5·3
Per cent survivors	64	47	55	57
First broods				
No. chrom.	457	1,083	774	988
No. leth.	19	3	44	3
Per cent leth.	4·16	0·27	5·68	0·34
Second broods				
No. chrom.	370	679	630	523
No. leth.	7	1	27	0
Per cent leth.	1·89	0·15	4·28	0·00

No. chrom., total number of chromosomes examined.
No. leth., number of lethal chromosomes.
Per cent leth., percentage of sex-linked recessive lethal mutations.
*r*A, adenosine; *r*H, inosine; *r*A5′-P, adenosine-5′-phosphate; *r*H5′-P, inosine-5′-phosphate.

Table 1 illustrates the results obtained when newly hatched larvæ are treated on the formaldehyde-containing synthetic treatment medium for 72 hr., and then transferred on to ordinary medium to complete their development. The presence of adenosine (*r*A) and adenosine-5′-phosphate (*r*A5′-P) in the treatment medium produces a high mutagenic activity of formaldehyde. However, if either inosine (hypoxanthine ribonucleoside, *r*H) or inosine-5-phosphate (hypoxanthine ribonucleotide, *r*H5′-P) is substituted for the corresponding adenine derivative, no induced mutagenic activity is detected. In comparing these two series of experiments, three differences should be noted. Thus, chemically, the only difference between the series is that the amino-group at the 6-position of the purine ring in adenosine and adenosine-5′-phosphate is replaced by a hydroxyl group in inosine and inosine-5′-phosphate. Biochemically, the adenine derivatives enter the organism with the correct purine groupings for utilization in nucleic acid synthesis, whereas inosine and inosine-5′-phosphate have first to be aminated before utilization in nucleic acid synthesis; since this amination takes place intracellularly a reaction-product of formaldehyde with these hypoxanthine derivatives in the treatment medium is therefore not formed. Biologically, the developmental response of *Drosophila* larvæ to the adenine and hypoxanthine derivatives is approximately equal; this suggests that they are utilized by the organism to approximately the same extent, and that the hypoxanthine derivatives are incorporated into the nucleic acids as adenylic acid, since this nucleotide component is the only one utilized to any appreciable extent. It has been shown[6] that when pyrimidines are excluded from the diet of developing *Drosophila* *melanogaster* larvæ, the development response to inosine is virtually the same as that of adenosine.

Table 2. Effect of Guanosine-3′-phosphate, Uridine-3′-phosphate, and Cytidine-3′-phosphate on the Mutagenic Activity of Formaldehyde, when Newly-hatched Larvæ are treated for 72 hr.

Treatment medium	*r*G3′-P + *r*U3′-P + *r*C3′-P	*r*G3′-P	*r*U3′-P	*r*C3′-P
Molarity of formaldehyde	$1{\cdot}0 \times 10^{-2}$			
Total larval developmental time in days	6·0	6·0	5·5	5·8
Per cent survivors	72	81	78	72
First broods				
No. chrom.	580	412	422	468
No. leth.	1	1	0	2
Per cent leth.	0·17	0·24	0·00	0·43
Second broods				
No. chrom.	470	493	417	462
No. leth.	1	0	1	0
Per cent leth.	0·21	0·00	0·24	0·00

*r*G3′-P, guanosine-3′-phosphate; *r*U3′-P, uridine-3′-phosphate; *r*C3′-P, cytidine-3′-phosphate.

It has been previously claimed[1] that the ribonucleoside-3′-phosphates of guanine, uracil and cytosine do not mediate the mutagenic activity of formaldehyde. Since the improved concentration for formaldehyde has been introduced, these compounds have been re-tested under the improved conditions; as seen in Table 2, the original findings were confirmed. In examination of the patterns of utilization of the ribonucleotide-3′-phosphates as growth requirements in *Drosophila melanogaster* larvæ, it has been shown[6] that the ribonucleoside-3′-phosphate of guanine, uracil and cytosine are not utilized when supplied in the absence of adenosine-3′-phosphate; consequently, metabolites formed by these compounds after reaction with formaldehyde would not be utilized in any event in this system. These compounds would therefore not be expected to mediate the mutagenic activity of formaldehyde.

Table 3 illustrates the results obtained when adenine (A), adenosine (*r*A), adenosine-3′-phosphate (*r*A3′-P), adenosine-5′-phosphate (*r*A5′-P), deoxyadenosine-5′-phosphate (*d*A5′-P), and inosine-5′-phosphate (*r*H5′-P) are tested for their ability to mediate the mutagenic activity of formaldehyde. For each treatment, the ribonucleoside-3′-phosphates

Table 3. Effect of Adenine, Adenosine, Adenosine-3′-phosphate, Adenosine-5′-phosphate, Inosine-5′-Phosphate, and Deoxyadenosine-5′-phosphate on the Mutagenic Activity of Formaldehyde, when Newly-hatched Larvæ are treated for the Entire Larval Life

Treatment medium	A	*r*A	*r*A3′-P	*r*A5′-P	*r*H5′-P	*d*A5′-P
Molarity of formaldehyde	$1{\cdot}0 \times 10^{-2}$					
Total larval developmental time in days	5·0	4·5	4·7	4·9	4·3	5·3
Per cent survivors	54	53	46	51	63	45
First broods						
No. chrom.	685	732	1,378	1,331	1,413	874
No. leth.	1	49	56	58	4	10
Per cent leth.	0·15	6·69	4·06	4·36	0·28	1·14
Second broods						
No. chrom.	537	490	897	571	607	860
No. leth.	1	10	27	37	2	12
Per cent leth.	0·18	2·04	3·01	6·48	0·33	1·40

A, adenine; *r*A, adenosine; *r*A3′-P, adenosine-3′-phosphate; *r*A5′-P, adenosine-5′-phosphate; *r*H5′-P, inosine-5′-phosphate; *d*A5′-P, deoxyadenosine-5′-phosphate.

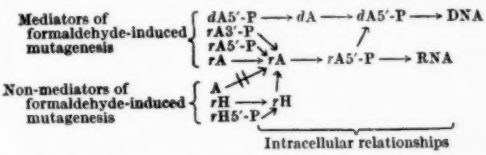
of guanine, uracil and cytosine are also added to the treatment medium, since an improvement in developmental time is found when these compounds are supplied as dietary supplements, though they are not otherwise concerned in the mediation of the mutagenic activity of formaldehyde (see Table 2). Adenine and inosine-5′-phosphate are seen not to be capable of mediating the mutagenic activity of formaldehyde, as expected from previous results with adenine[1] and inosine-5′-phosphate (Table 1). Adenosine, adenosine-3′-phosphate, adenosine-5′-phosphate, and deoxyadenosine-5′-phosphate are all found to mediate formaldehyde-induced mutagenesis; but differences are observed in the mutagenic response produced by these adenine derivatives. Adenosine produces a significantly greater response in the first brood than adenosine-3′-phosphate and adenosine-5′-phosphate, whereas the response in the second brood is significantly greater with adenosine-5′-phosphate than with adenosine and adenosine-3′-phosphate. Second broods are usually sampled to detect any increase in the speed of germ-cell development, where such an effect may increase the mutagenic response in the second brood. Since the formaldehyde-containing medium loses its mutagenic activity after 24–48 hr., an increase in germ cell development may result in an increased number of cells passing into the sensitive stage of the second brood during the period of effective treatment. However, this does not appear to be the sole factor responsible here as the adenosine-5′-phosphate culture has a slower overall larval development-time than the adenosine, adenosine-3′-phosphate and inosine-5′-phosphate cultures. Deoxyadenosine-5′-phosphate shows a significantly lower ability to mediate formaldehyde-induced mutagenesis than the adenine ribonucleotides; this may be because the concentration of deoxyadenosine-5′-phosphate used is not optimal for utilization by the larvæ. Data for the growth response of *Drosophila* larvæ to deoxyadenosine-5′-phosphate on the synthetic medium are not available.

As previously discussed[1], the mechanism of action of the postulated *AA* metabolite is mediated by way of deoxyribonucleic acid synthesis. However, the ribonucleotide derivatives of adenine (which are constituents of ribonucleic acid) are found to be more effective than the deoxyribonucleotide derivative (deoxyadenosine-5′-phosphate) tested, which is a constituent of deoxyribonucleic acid. Although relatively little is known of deoxyribonucleotide synthesis compared with that of ribonucleotide synthesis, it has been shown that ribonucleotides are effective deoxyribonucleic acid precursors[7].

It is thought that the synthesis of nucleotides follows a common pathway up to a point where a direct conversion of the ribo- to the deoxyriboform takes place by simple enzymic reduction of the ribose sugar.

The nucleoside-5′-phosphates are the natural nucleotide precursors for ribonucleic acid, deoxyribonucleic acid, and nucleotide coenzyme synthesis. The ribonucleoside-3′-phosphates (which are products of alkaline hydrolysis of ribonucleic acid) are incorrectly phophorylated for direct utilization in these syntheses. In *Drosophila* larvæ the adenine ribonucleoside-3′- and 5′-phosphates are utilized, however; but there is indirect evidence that dephosphorylation of these adenylic acids to adenosine precedes assimilation by the larvæ[6]. Thus, adenosine-5′-phosphate, adenosine-3′-phosphate, and probably deoxyadenosine-5′-phosphate would be converted to their nucleosides before assimilation. For reasons stated earlier, adenine, inosine, and inosine-5′-phosphate do not mediate the mutagenic activity of formaldehyde, although they are normally utilized by *Drosophila* larvæ. A schematic representation of these relationships is shown in Fig. 1.

Fig. 1. Schematic representation of the probable utilization by *Drosophila melanogaster* larvæ of the purines under consideration.



A, adenine; *r*A, adenosine; *r*A3′-P, adenosine-3′-phosphate; *r*A5′-P, adenosine-5′-phosphate; *r*H, inosine; *r*H5′-P, inosine-5′-phosphate; *d*A, deoxyadenosine; *d*A5′-P, deoxyadenosine-5′-phosphate; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

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IMPROBABILITY OF THE CHELATION HYPOTHESIS OF AUXIN ACTION

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THE suggestion has been advanced on biological grounds that plant-growth regulators exert their physiological effects through complex formation with certain cations, such as one or more of the heavy metals[1]. For example, with indole-3-acetic acid (IAA), chelation has been postulated to involve either the carboxyl group and the aromatic ring[2] or the imine and the carboxyl groups[1]. Because chemical evidence for any such mode of action has been scanty and conflicting, and no quantitative stability constant data for aqueous solutions have been published, the present definitive study was undertaken.

Cupric ion is the obvious choice for experiments aimed at providing such evidence because, of all the cations normally found in plant tissue, it forms by far the most stable series of complexes with all known ligands. Cohen *et al.*[2] found the absorption spectrum

of IAA in 50 per cent aqueous ethanol was changed when copper nitrate was added: this they attributed to the formation of 1 : 1 and 1 : 2 copper complexes which, from *p*H titration data, had stability constants of $10^{2.2}$ and $10^{5.4}$. Recaldin and Heath[3], repeating this work, were not confident of the interpretation of the observed changes; in addition, they found the stability constant of the 1 : 2 complex to be $10^{3.0}$, which is about 1/250 of the previous value. Afterwards, Fawcett[4] showed that in the experimental conditions a major contribution to the results came from hydrolysis of free cupric ion, a factor that had not been considered. Armarego *et al.*[5] failed to find any correlation between the growth activity of phenoxyacetic acids and the stability constants of their copper complexes.

All the foregoing results were obtained using 50 per cent ethanol solutions and hence should not be applied to interpret observations made on purely aqueous media, especially as complex formation between IAA and metal ions *in vitro* in water could not be demonstrated by either Cohen *et al.*[2] or Recaldin and Heath[3]. This failure was presumed to be due to the attainable concentrations of free IAA being too low. The poor solubility of IAA in water precludes the use of the Bjerrum *p*H-titration method of stability constant determination, which is based on the displacement of a proton from the free acid by the cation which forms the complex. A further complicating factor is the hydrolysis of cupric ion, which involves a polynuclear process[6] so that its extent varies with the copper concentration as well as with the *p*H, making it necessary to work either at low *p*H values or at low copper concentrations (or both). This limits the usefulness of spectrophotometric methods. However, the copper-amalgam electrode[7] can be made the basis of a sensitive method which is free of these defects and which has been used in the present instance. The potential of this electrode depends on the activity of cupric ion in the solution. If a high concentration of an inert supporting electrolyte, such as potassium nitrate, is also present, the activity coefficient of cupric ion remains constant so that when a complexing species is added to the solution the potential measures directly the free ion concentration. Knowing this quantity, as well as the total copper and ligand concentrations, enables the stability constant of the complex to be calculated.

Experimental conditions (solution about *p*H 5 and initial copper concentration of 5×10^{-4} *M*) were chosen so that hydrolysis of copper ion was negligible[6], while, at the same time, advantage could be taken of the much greater solubility of the anions of the acids tested. The self-buffered solutions of IAA and α-naphthylacetic acids that were added were 1 *M* in potassium nitrate and 0·025 *M* in the acid plus its potassium salt; for the other acids a concentration of 0·05 *M* was used. Even at these concentrations, which in some cases represented supersaturated solutions, only very small changes in potential were observed and great care was needed for their measurement against a saturated calomel electrode. For this reason a potentiometer (Tinsley vernier) and galvanometer (Cambridge spot) combination was selected which could be read easily to 0·02 mV., and a Vibron electrometer *p*H meter with attached recorder was used to provide a continuous check on the *p*H of the solutions. The copper–amalgam surface was freshly exposed and solutions were kept at 20·0° C. in an atmosphere of nitrogen.

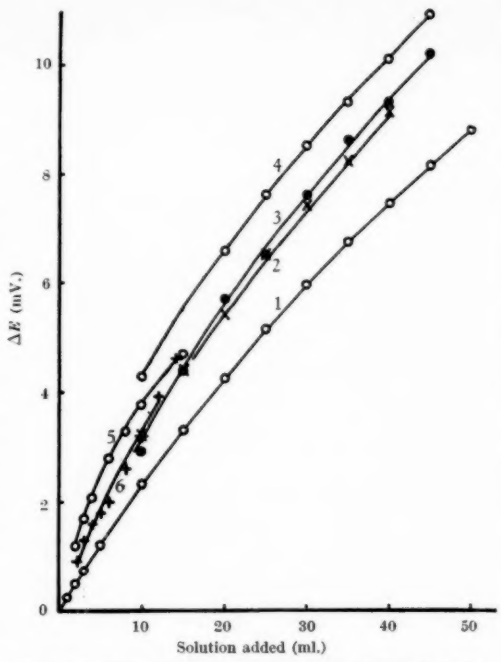

Fig. 1. Change in copper–amalgam electrode potential at *p*H 5·0 when 50 ml. 5×10^{-4} *M* copper nitrate in 1 *M* potassium nitrate was diluted with: (1) 1 *M* potassium nitrate; (2) 0·05 *M* 2,4-dichlorophenoxyacetic acid buffer; (3) 0·05 *M* 2,4-dimethylphenoxyacetic acid buffer; (4) 0·05 *M* 4-chlorophenoxyacetic acid buffer; (5) 0·025 *M* indole-3-acetic acid buffer; (6) 0·025 *M* α-naphthylacetic acid buffer. Solutions 2–6 were also 1 *M* in potassium nitrate

From the small but reproducible differences shown in Fig. 1 the stability constant, $K_1 = (\mathrm{Cu}.L^+)/(\mathrm{Cu}^{++})(L^-)$, where (L^-) is the anion concentration of the complexing species, has been calculated for the 1 : 1 complexes of each of the acids tested. The results set out in Table 1 show log K_1 to range from 0·8 to 1·7. This indicates that no greater degree of complex formation occurs than is known to exist in the cupric salts of simple carboxylic acids such as acetic and chloroacetic[8]. The slight increase of log K_1 values with the pK_a of the acids reflects only the commonly observed general parallelism between the strengths of binding of metal ions and protons in any related series of ligands. No evidence was obtained for the formation of 1 : 2, or higher, complexes, in agreement with the usual observation for many acids that log K_2 of their complexes is much less than log K_1.

The stability constants of the copper complexes of IAA and acetic acid are almost identical and, as the pK_a values of the two acids are comparable, the conclusion is inevitable that in IAA, also, only the carboxyl group plays any part in complex formation. For this reason, it appears justifiable to take the stability constants of complexes of other metals with IAA to be the same as for the corresponding acetates[9]. For zinc-, calcium- and magnesium-IAA complexes the constants would be about 1/10, 1/40 and 1/40, relative to copper.

Although the present results show the plant-growth acids of the types investigated to be only weak complexing agents, it is of some interest to assess the maximum possible extent to which they might form

Table 1. IONIZATION CONSTANTS OF SOME PLANT-GROWTH REGULATORS AND THE STABILITY CONSTANTS OF THEIR COPPER COMPLEXES

Acid	pK ($\mu = 0{\cdot}0005$)	$pK_{conc.}$ ($\mu = 1$, KNO_3)	log K_1 (copper complex)
2,4-Dichlorophenoxy-acetic	2·90	2·78	0·8
4-Chlorophenoxyacetic	3·00	2·91	1·1
2,4-Dimethylphenoxy-acetic	3·22	3·08	0·9
α-Naphthylacetic	4·22	4·04	1·4
Indole-3-acetic	4·54	4·36	1·7
Chloroacetic (ref. 8) (for comparison)		2·66	0·91
Acetic (ref. 8) (for comparison)		4·61	1·65

metal complexes under biological conditions, taking for this purpose the ions Cu^{++} and Zn^{++}. Based on figures for metal–acetic acid complexes[9], the reasonable assumption is made that changing the ionic strength of solutions of 1·0–0·15 only slightly increases log K_1.

The total copper concentration in fresh plant tissue does not often exceed 1 p.p.m., that is, 17 μM. Young and growing tissue contains a higher free amino-acid concentration than mature tissues[10,11], but, even in the latter, total free amino-acid concentrations in excess of 5×10^{-3} M have been found in normal bracken, spinach beet and pea leaves[11]. It can readily be calculated from the latter figure (using 9·5 as the average pK of amino-acids, and 8·5 and 15·5 as the average log stability constants of their 1 : 1 and 1 : 2 amino-acid complexes at 20° (ref. 9)) that at pH 7 the free cupric ion concentration in plant tissues must be less than 10^{-11} M. Even then, this figure takes no account of the presence of competing ligands such as proteins, which must reduce the free copper concentration even further. Growth-stimulation has been demonstrated at concentrations of 10^{-9} M IAA[1], which, from the above results, must represent a copper–IAA complex concentration of less than 10^{-18} M. For similar calculations with zinc, an average zinc content of 3 mgm./kgm. green tissue has been taken. (This is an arbitrary figure because the zinc content of plants varies considerably with differences in soil zinc-level, but, from a summary of the literature[12], 1–10 p.p.m. seems to be the normal range). Taking an average log stability constant of 5·3 for 1 : 1 zinc–amino-acid complexes[9] leads to the conclusion that the concentration of zinc–IAA complex in plants must be less than 10^{-14} M. Both this and the copper value are so very much less than for any known biologically active substances as to make it extremely probable that metal-complex formation plays no part in the physiological activities of IAA and the other plant-growth regulators discussed above.

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ENVIRONMENTAL FACTORS AND BRYOPHYTES

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MOST plants appear to show a definite rhythm or periodicity in their vegetative growth, and to a more marked extent in their reproduction, connected with a periodicity in some factor or factors of their environment.

As early as 1925, Wann[1] suggested that all plants (with the possible exclusion of fungi and lichens) can be grouped into 'long-day' and 'short-day' plants depending on the photo-period or length of daily illumination which results in the production of 'flowers' and 'fruit'. Since then, the responses of higher plants to photo-periods, temperature, and auxins or growth substances and the interactions of these factors have been increasingly investigated. Relatively little, however, has been accomplished in the field of mosses and liverworts, probably because of the slight economic importance of these plants.

This article aims at reviewing some of the preliminary experimental work which has been performed with these groups, with particular reference to the environmental factors that influence vegetative growth and induce the sexual or sporangial condition.

Wann initiated work in this field by carrying out photo-periodic experiments using *Marchantia polymorpha* as his material, and his results indicated that *Marchantia* appeared to respond to photo-periods as do long-day plants in the higher plants. Voth and Hamner[2] repeated these experiments under improved conditions and again demonstrated certain definite photo-periodic responses in both vegetative thalli and sex organs, in both long- and short-day conditions, irrespective of the nutrient supply: (*a*) plants under long-day conditions were always larger and had greater dry weight than plants on comparable solutions under short-day conditions; (*b*) plants under long-day conditions produced a greater number of gametangiophores than similar plants on comparable solutions under short-day; (*c*) plants under short-day conditions produced a greater total number of gemmæ cups than plants on comparable solutions under long-days; (*d*) it was also noted that low humidity retarded the responses especially of the female branches and organs, whereas high humidity hastened the response.

Similar experiments were carried out at Cardiff using three other genera of the Marchantiales. In view of the results of Voth and Hamner, no attempt was made to culture plants on specific media, but turfs growing on their natural substrate were collected from the field. The experimental chambers were illuminated by six 4-ft., 40-W. fluorescent tubes supplemented by two red fluorescent tubes to make up the deficiency in waves from the red end of the spectrum. Heating was performed with a diffused heating spiral, and temperature was controlled by a cartridge thermostat to within ± 1° F. Intensity of light was about 800–1,000 ft.-candles at a distance of 1 ft. from the light source and measured at the surface of the plants. Day-length was controlled by Venner time switches. A second series of cabinets showed a greater fluctuation of temperature as they were not heated independently.

In all the species investigated, increased vegetative growth occurred in the long-day chambers, and sexual branches were stimulated on these plants; the plants under short-day conditions remained vegetative (Table 1).

Table 1

Species	Long-day				Short-day			
	Plants used		Plants responding		Plants used		Plants responding	
	♂	♀	♂	♀	♂	♀	♂	♀
Marchantia polymorpha	62	91	35	58	51	56	0	3
Preissia quadrata	62	177	41	141	40	72	0	0
Conocephalum conicum	94	119	88	89	52	61	0	0
Lunularia cruciata	85*	103	47*	86	38	67	0	2

* Denotes field observations (August).
Average maximum temperature, 65° F.; average minimum temperature, 46° F.

The response of *Marchantia polymorpha* to increased light intensity was checked. It was found that plants in experimental chambers having supplementary lighting always responded more rapidly than those in a lower light intensity, but that day-length influenced the actual number of gametangiophores that were produced (Table 2). This would appear to be comparable with the reactions of higher plants, since Mann[3] demonstrated a similar effect on floral initiation in *Xanthium*, where increase in light intensity increased the effect of the photo-period.

Table 2. Photo-periodic and Light Intensity Effects in the Marchantiales
No. of gametangiophores produced in response to differing light intensities

Period of response (weeks)	14 hr. (500 ft.-candles)	14 hr. (1,000 ft.-candles)	18 hr. (1,000 ft.-candles)
1	0	0	0
2	0	3	30
3	0	7	35
4	2	7	35

Average maximum temperature, 76° F.; average minimum temperature, 58° F.

The response of *Lunularia cruciata* was of particular interest, since sexual reproduction in this form only occurs sporadically in the British Isles and in many other countries.

Experimentally, archegoniophores were only induced to develop during the months of December to March inclusive, when plants from the field, transferred to a temperature of 70° F. and long-day conditions, would produce archegoniophores within 7–8 days[4]. A temperature as low as 60° F. has been found to be just as effective early in the year and, owing to the rapidity with which *Lunularia* can produce sexual organs, the temperature stimuli need only be maintained for a few days. Later collections, that is, after March, remained vigorously vegetative, so that apparently there may be a low-temperature requirement which must be fulfilled before the stimulus of a rise in temperature. Other evidence of this is shown by plants grown from gemmæ, for these do not produce archegoniophores until the following spring, having first passed through the cold period of winter. Plants suffering from frost damage do not produce sexual branches; their apices give vegetative growth throughout the following season. Only plants kept at low temperatures, but protected from frosts, give rise to branches with reproductive organs when submitted to a rise in temperature and long days. Male branches have not been stimulated experimentally, but field records suggest that a summer temperature of 70–80° F. is necessary for their initiation.

Lunularia contrasts strongly with *Conocephalum conicum*, a native of Britain, which dies back in winter and regenerates from the apex each spring. It has been found that when dormant clones of *Conocephalum conicum* are transferred to growth chambers at 70° F. and long days, vigorous vegetative growth occurs, but after 2 weeks the apices begin to produce sexual branches. Clearly in *Conocephalum*, the death of the older parts of the thallus does not affect the ability of the regenerated branches to produce sexual organs.

Temperature apparently did not affect the response in this species or in *Preissia quadrata*, where the stimulus of long days is wholly responsible for the re-orientation of the growth of the thallus from the horizontal to the vertical plane, which is the first step to the production of the sexual receptacles. It would appear that this re-orientation is probably correlated with the synthesis of growth hormones, or the removal of a growth inhibitor, in the long days, since Rousseau[5] apparently demonstrated that the external application of auxins (2,4-dichlorophenoxyacetic acid, α-naphthalene-acetic acid, and β-naphthoxyacetic acid) to the vegetative plants of *Marchantia polymorpha* induced morphology comparable to the receptacles of normal plants. I have also induced a similar re-orientation of the thallus with receptacle formation in *Conocephalum conicum* using 2,4-dichlorophenoxyacetic acid, at a concentration of 10^{-4}, but no sex organs were formed. Other experiments indicated that application of high auxin concentrations to the normal young receptacles of these species in early summer inhibited the formation of sex organs. These observations are of interest in view of the work of Salisbury[6], who, among others, has demonstrated that the flowering of many higher plants is influenced by the application of auxin. High concentrations were inhibitory to the flowering, not only of short-day plants, but also of many species including long-day and indeterminate forms.

Normally the female receptacles remain very shortly stalked until fertilization has occurred. In *Conocephalum conicum* this occurs in the field in June, and the sporogonia develop soon after, but the stalk of the archegoniophore does not elongate until the following spring, usually from March onwards into the summer. Elongation of the stalk occurs by extension of the individual cells comprising the stalk and not by cell division.

Table 3. Effect of Varying Concentrations of 2,4-Dichlorophenoxyacetic Acid on the Archegoniophore Stalks of *Conocephalum conicum*

Days after treatment	Concentration of 2,4-D					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Control
	Average length of archegoniophore stalks in mm.					
7	2·0	1·5	2·0	2·0	1·5	2·0
14	5·0	11·0	11·0	4·0	2·0	2·0
21	5·0	11·0	11·0	12·0	2·0	2·0
28	5·0	11·0	11·0	18·5	2·0	2·0

Average maximum temperature, 62° F.; average minimum temperature, 58° F. Normal day-length for October–November.

We have[7] stimulated elongation in the archegoniophore stalks of *Conocephalum conicum* in October, by the external application of 2,4-dichlorophenoxyacetic acid, greatest elongation occurring at a concentration of 10^{-6} (Table 3). This suggests that normal elongation is brought about by a natural auxin produced within the gametophyte. As normal elongation occurs in the spring, the production of auxin may be stimulated by the greater temperature and photo-period of the spring days. To ascertain whether increased photo-period alone would stimulate endogenous auxin production, and thus bring about elongation, portions of the female thallus bearing archegoniophores were subjected to long-day conditions (16 hr.) at normal winter temperatures (January). No elongation occurred and so it was assumed that photo-period alone was not sufficient to stimulate auxin production, or at least in sufficient quantity to bring about elongation of the cells. A second group of plants was subjected to long-day treatment in a heated greenhouse at an average temperature of 60° F., when elongation of the stalks occurred (Table 4). Higher temperatures had a retarding effect, as did short days at both temperatures, indicating that the long photo-period combined with a temperature of 60° F. was optimal for cell elongation. This appears to be an effect similar to that observed by Knott[8], when studying the effect of temperature on the photo-periodic response of spinach. With the photo-period fixed at 15 hr./day, seed stalk elongation occurred sooner if temperatures during treatment were held at 60–70° F. than it did if held at a higher or lower range.

Table 4. Effect of Photo-period and Temperature on Elongation of the Archegoniophore Stalk of *Conocephalum conicum*
Responses given for 5 archegoniophores after 4 weeks

Temperature		16 hr.		8 hr.	
Maximum	Minimum	Average length	No. responding	Average length	No. responding
40° F.	34° F.	2·0 mm.	0	1·5 mm.	0
62° F.	58° F.	19·0 mm.	5	1·5 mm.	0

That a natural auxin is present in the thalli has been demonstrated by Dickson[9], and Larue and Narayanaswami[10], who obtained positive results with *Avena* tests of the extracts of thallus tips of *Lunularia* and *Marchantia*. Preliminary experiments with analysis of such extracts by paper chromatography suggest that the auxin involved may be indole-3-acetic acid.

The sporophytes of the Marchantiales are small and more or less parasitic on the gametophyte, although some chlorophyll is formed. Hughes[11] has shown that in *Conocephalum conicum* sporophytes are complete in all their organs, 8 weeks after fertilization, but the slight elongation of the seta, typical of liverworts, had not occurred. With the temperature maintained at 70° F., half the plants were placed in long-day, and half in short-day, conditions. The development of the sporophytes was not found to be influenced by day-length.

Seta elongation has been induced by external application of auxin to young sporophytes of *Conocephalum conicum* in early autumn. This elongation was not influenced in any way by differences in day-length, and although capsules dehisced normally the spores were not viable.

Of the seven species so far investigated in the Jungermanniales, six appear to react as long-day plants, the sexual organs being stimulated to form in December and January, on plants placed in experimental chambers in long days, with a maximum temperature of 65° F. and a minimum of 46° F. Plants in short days remained vegetative at similar temperatures (Table 5).

Table 5. Photo-periodic Responses in the Gametophyte of Some Jungermannials

Material	16 hr.				8 hr.			
	Plants used		Plants responding		Plants used		Plants responding	
	♂	♀	♂	♀	♂	♀	♂	♀
Dioecious forms								
Riccardia pinguis	28	70	11	34	14	29	0	0
Cryptothallus mirabilis	5	10	3	9	5	10	2	7
Diplophyllum albicans	157		66	38	184		0	0
Monœcious forms								
Riccardia multifida	79		52		67		0	
Pellia epiphylla	76		44		82		0	
Lophocolea cuspidata	124		83		137		0	
Lophocolea heterophylla	115		78		121		0	

Average maximum temperature, 65° F.; average minimum temperature, 46° F., December–January.

Of interest in this group is the behaviour of *Cryptothallus mirabilis*. In Nature, the plants are found up to approximately 3 in. below the surface of leaf litter or a moss carpet overlying peat or humus. The thallus is often heavily infected with fungal hyphæ, which destroy the chlorophyll in many of the cells. The apices of young branches from which the sex organs arise are usually free from fungus. In this form the sexual organs are not produced in response to a particular day-length, but to a rise in temperature.

Experimentally, the organs formed in both long- and short-day conditions within 5 weeks of the plants being transferred from the field to the higher temperatures of the experimental chambers. Plants kept continuously in a warm greenhouse at 65° F. remained vegetative throughout the winter and the following season, suggesting that here, as in *Lunularia*, there may be a low-temperature requirement that must be fulfilled before the stimulation of a rise in temperature.

As in Marchantiales, maturation of the sporophytes is independent of day-length, but Crombie and Paton[12] have demonstrated that temperature affects seta elongation. They have shown that older sporophytes of *Pellia epiphylla*, left in the field until January or February, respond more rapidly to a temperature stimulus in the laboratory than do sporophytes collected in early autumn. They suggest that during the winter there is a slow synthesis of growth hormones (or removal of a growth inhibitor), which is stimulated to some extent by temperature and extra light. That temperature may well be the more important factor is indicated by the results of the experiments I have carried out in November on plants

kept at an average temperature of 60° F. Application of various concentrations of 2,4-dichlorophenoxyacetic acid to plants bearing young sporophytes caused seta elongation to occur in the three liverworts investigated, maximum extension occurring at a concentration of 10^{-6} (Table 6). A study of seta elongation by time-lapse photography has also indicated that the extension of individual setæ of these genera often occurs over a period of two or three days, and that there is a period of growth immediately following sunset each day (Fig. 1). It is realized that growth could have been influenced by the light flashes used for the photographs, but if this light source were significant, one would have expected a more marked effect on the growth curves obtained.

Table 6. Effect of Varying Concentrations of 2,4-Dichlorophenoxyacetic Acid (2,4-D) on Seta Elongation in Some Jungermanniales

Species	Concentration of 2,4-D					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Control
	Average length of setæ in mm.					
Pellia epiphylla	5·0	13·8	14·5	28·5	24·0	1·0
Cryptothallus mirabilis	1·0	4·0	16·0	18·6	17·2	1·0
Lophocolea cuspidata	0·5	0·5	12·5	22·5	21·0	0·5

Results after 14 days.
Average maximum temperature, 60° F.; average minimum temperature, 58° F., November–December.

Experiments by Asprey *et al.*[13] also have shown that external application of indole-3-acetic acid can produce seta elongation in young sporophytes of *Pellia*, and that addition of gibberellin gives greater extension than with the auxin alone—an average of 63 mm. in length as compared with 37 mm. in the former case.

Brian and Hemming[14] have presented evidence that gibberellin often depends on the presence of indole-3-acetic acid for its activity. Brian[15] suggests that gibberellin is produced naturally in plants in response to light, vegetative growth being proportional to the amounts of gibberellin and indole auxins which are formed. He suggests that this may well be true of the extension of *Pellia* setæ, and that gibberellins formed during daylight are utilized in the dark period.

Hughes carried out experiments on *Anthoceros laevis* in April and May when plants from the field had already discharged their antheridia and young sporophytes were formed on the dorsal surface of the thalli of the female plants.

Plants were placed in experimental chambers under long- and short-day conditions at temperatures of 70° F. and 50° F. in each case. After a month,

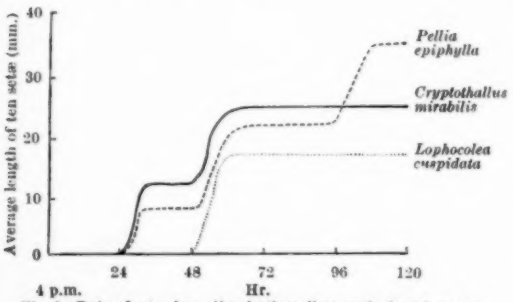

Fig. 1. Rate of seta elongation in three liverworts, based on time-lapse photographs 55° F.; hr. of darkness, 5.30 p.m.–8.0 a.m.

Table 7

Anthoceros laevis	18 hr.		6 hr.	
	70° F.	50° F.	70° F.	50° F.
Male plants	vegetative	vegetative	fertile	fertile
Female plants	vegetative	vegetative	fertile	fertile
Sporophytes	rapid vegetative growth	slow vegetative growth	slow vegetative growth	slow vegetative growth

the male plants were sexually fertile in short days at both temperatures, the antheridia developing on new lobes of the thalli. Plants under long-day conditions remained sterile. Subsequent experiments have shown similar results for the female plants (Table 7).

When investigating the responses of the sporophytes, Hughes was able to show that after 8 weeks those sporophytes which had been maintained under long-day conditions exhibited almost unlimited power of vegetative growth due to the basal meristem, as compared with the low vegetative growth in short days. There was no difference, however, in the rate of spore production in long and short days. It was concluded that development of the sporophyte in this species is not induced by the photo-period except for a difference in the rate of the two sporophytes vegetatively. The length of the photo-period did not affect the embryonic tissue.

In the Sphagnales, as yet, I have made only a few preliminary observations on *Sphagnum plumulosum*. Material collected in August and kept in a glasshouse in short-day conditions produced sex organs in December. The male branches changed colour as they matured and appeared as red catkins in early February when the antheridia dehisced. Dissection of the apices of branches of the female plants showed that archegonia were present. Plants in experimental chambers under long-day conditions did not produce sex organs (Table 8).

Table 8

Material	16 hr.				8 hr.			
	Plants used		Plants responding		Plants used		Plants responding	
	♂	♀	♂	♀	♂	♀	♂	♀
Sphagnum plumulosum	134	115	0	0	110	107	54	31

Average maximum temperature, 76° F.; Average minimum temperature, 58° F.

In the field, plants were producing sex organs in late February and early March, capsules had formed by May, but these did not mature and dehisce until July. As yet there is no experimental data concerning the effect of temperature or day-length on the maturation of the sporophyte. The only record of auxin action in this group is the work by Patterson[16], who demonstrated that nearly mature capsules with unelongated pseudopodia gave maximum extension within 3–4 days when moistened with indole-3-acetic acid at a concentration of 10^{-6}. Higher concentrations of auxins, however, inhibited or entirely prevented elongation, a reaction similar to that recorded for the receptacles and stalks of the Marchantiales.

There is therefore some evidence that *Sphagnum plumulosum* may well be a short-day plant, but more confirmation is required.

Of the Bryales or true mosses, the only experimental work so far has been carried out by Hughes using *Polytrichum aloides* and *P. piliferum*.

In *Polytrichum aloides* mature sex organs are produced in the field from March until May, but arche-

gonial production frequently continues until July, particularly on plants where fertilization has failed. In the latter circumstances the number of archegonia produced is frequently increased to double the normal number. Where sporophytes have formed these are usually apparent by the end of July, covered by the white calyptra. The capsule takes shape in August and September, is fully formed by December, and matures to dehisce in March or April (Table 9).

Table 9. FIELD OBSERVATIONS OF THE MATURATION CYCLES OF *Polytrichum* SPECIES (J. G. HUGHES, 1955)

Developmental phase	*P. aloides*	*P. piliferum*
Sex organs mature	Early March	April
Vegetative phase of sporophyte	Short	Long
Onset of reproductive phase of sporophyte	May	November
Mature sporogonia	August	Following March
Capsules dehisced	Following spring	Early summer

Polytrichum piliferum

Date	Average length of apical meristem (cm.)	Developmental phase
Oct. 5	0·030	Vegetative, endothecium unsegmented
Oct. 23	0·035	Apical cell ceased to divide; endothecium segmented
Nov. 23	0·060	Endothecial block starts to swell at base; apical cell begins to vacuolate
Dec. 21	0·070	Little change
Jan. 18	0·085	Operculum and theca clearly demarcated
Feb. 15	0·110	Archesporial layer differentiated
Mar. 15	0·150	Archesporial tissue several cell layers thick; peristome apparatus developing
April 12	0·450	Sporangium fully developed

Female plants placed in experimental chambers in January under long- and short-day conditions, combined in each case with temperatures of 70° F. and 50° F., produced archegonia within two weeks in both long and short days, about 66 per cent responding in each case. Even after a month the plants in both cabinets at 50° F. were still vegetative (Table 10). From these results, it was concluded that day-length was not the governing factor in the production of sex organs in *Polytrichum aloides*, but that the rise in temperature in the spring months in the field may be the most important factor causing the sudden appearance of sex organs.

Table 10. PHOTO-PERIODIC RESPONSES IN *Polytrichum aloides* (J. G. HUGHES, 1955)

Period of response (days)	18 hr.		6 hr.	
	70° F.	50° F.	70° F.	50° F.
14	66 per cent response	—	66 per cent response	—
28	—	No response	—	No response

Twenty-five gametophytes (female) used in each case. Experiment conducted in January.

A second experiment was set up involving diurnal fluctuations of temperature in combination with the two photo-periods. By these experiments it was hoped to indicate whether the temperature stimulus was a light or dark reaction. The results indicated that there was a slightly greater response in the warm nights than in the cold (Table 11). A temperature stimulus of 20° F. proved sufficient to induce the development of archegonia in the laboratory two months before their earliest appearance in the field.

The sporophyte grows by means of an apical cell until a branch is formed. This does not enter into the reproductive stage until the apical cell loses the power to divide, and the whole original meristem is then incorporated into the developing sporangium.

Table 11

Response (per cent)	Light (hr.)	Temperature of light period (°F.)	Dark (hr.)	Temperature of dark period (°F.)
54	18	70	6	50
70	18	50	6	70
44	6	70	18	50
60	6	50	18	70

Plants of *Polytrichum aloides* bearing vegetative sporophytes were placed in experimental chambers in June, in 8 hr. and 18 hr. light respectively at normal temperatures for the time of year. At intervals of 3, 5 and 7 weeks, the cultures were exchanged and, once changed, remained in that cupboard up to a total period of 24 weeks. By sectioning ten sporophytes from each group, and measuring the total seta length and total meristem length, it was found that short days stimulated development of the capsule and spore production. Long days did not entirely inhibit spore production, but did have a distinct retarding effect on sporangium development. The length of the seta was also found to be influenced by the photoperiod, since in short days the vegetative stage is very limited, giving rise to a short seta, whereas in long days there is a late change to the reproductive phase and so a long seta is formed. To ascertain whether this was due to an increase in the number of cells, or to cell length, the setæ of both long- and short-day sporophytes were macerated and measured. Cells from the central strands were used as these were not as spiralled as the outer cells of the setæ. An average of 200 cells of each kind of seta (Table 12) indicated that it is a difference in cell length and not in cell number which causes the difference in seta length. It was concluded that *Polytrichum aloides* is only facultatively photo-periodic in spore production since, although spore production is markedly promoted in short days, it can also be finally achieved even in long days.

Table 12

Day-length	Response	Average length of 200 cells in seta (arbitrary units)
18 hr.	Slow*	3·71
6 hr.	Rapid*	2·61

* The true vegetative state is taken when the average meristem length is 0·04 cm.; the true reproductive state is taken when the average meristem length is 0·114 cm.

Polytrichum piliferum differs in that the sporophytes do not mature until March of the following year, so that sporangia pass through a cold winter period (in contrast to *Polytrichum aloides* where sporangia are mature in August of the same year) (Table 9).

Plants bearing vegetative sporophytes were removed from the field at monthly intervals, to growth chambers at 70° F., half being submitted to long days and half to short days, the field behaviour of the plants being studied as a control. Plants from collections made in October did not produce sporangia in the experimental chambers, although apical growth had ceased and some segmentation had occurred. This suggests that the character of the sporophytes as a branch of limited growth is inherent in the sporophyte itself and not contingent on environmental factors. Collections made on November 23 and afterwards, gave rise to small, normal capsules in both day-lengths. It was concluded that sporangium

development was indifferent to day-length and that *Polytrichum piliferum* probably has a low-temperature requirement in its spore production which is normally satisfied in the field from late November onwards.

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CARBON DIOXIDE AND ETHANOL AS FACTORS CONTROLLING THE GROWTH OF ETIOLATED OAT SEEDLINGS

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WHEN the airstream passing over etiolated oat seedlings is enriched with carbon dioxide to a concentration of 5 per cent for 3 days from the time of planting, then during the 5 days following cessation of treatment with carbon dioxide, the growth of the seedlings is modified in the following respects: (1) the mesocotyl, after an initial depression, shows a marked promotion[1–3]; (2) the coleoptile is depressed[1–3]; (3) the amount of reserves transferred from the endosperm to the embryo is reduced[2,6]; and (4) the mesocotyl retains a capacity to elongate after a brief exposure to light[4,5]. In the absence of carbon dioxide in the airstream, identical growth variations can be induced to occur during the interval mentioned, by treating the seedlings with 0·3 per cent ethanol through the roots for 3 days from the time of planting (references as above) and further, when the ethanol and carbon dioxide treatments are administered simultaneously, their effects on mesocotyl growth are independent and additive[4].

The coincidence between these effects on growth suggests that a single phenomenon may be under investigation, that is, the ethanol absorbed from the culture medium (London tap-water) might give rise *in vivo* to carbon dioxide to which both effects should be attributed; or, alternatively, treatment with carbon dioxide might induce ethanol formation, perhaps by occasioning a fermentative change in metabolism; in this event the growth responses observed should be regarded as ethanol effects.

The data to be presented favour the first alternative.

The effect of treatment with ± 5 per cent carbon dioxide combined factorially with ± 0·3 per cent ethanol on the ethanol content of 3-day-old seedlings has been determined on 10 replicate samples, each of 5 plumules, by distilling the volatile constituents under reduced pressure into an aliquot of acid dichromate and titrating the residual dichromate with sodium thiosulphate.

The mean ethanol contents, in μgm. per 5 plumules, are recorded in Table 1, exp. 1. It will be seen that treatment with ethanol alone increased the content in the plants, but the overall effect of ethanol on the internal content, as shown in the analysis of variance, did not reach significance. This was due to the highly significant negative interaction with carbon dioxide, which when administered alone reduced the amount of ethanol in the plumules but apparently did so more effectively when ethanol was additionally supplied.

Because of the variation in growth with treatment, determination of the ethanol in a given number of plumules means that different weights of material were assayed. As these samples were collected in darkness no correction could be made for the differences, but in a further experiment, the 5 plumules in each sample were briefly exposed to light for measurement. The ethanol contents were determined and submitted, as previously, to an analysis of variance (Table 1, exp. 2). Additionally, the total lengths of the mesocotyl + coleoptile + leaf of the 5 plumules in each treatment are quoted in italics, together with an analysis of covariance. From these length-measurements the content of water was estimated so that the concentration of ethanol in the plumules could be assessed. The content in μgm. and analysis of variance agreed with those of exp. 1, but the analysis of covariance shows that by allowing for variation in length, the depressive effect of carbon dioxide on the ethanol content of the seedlings was eliminated while the levels of significance due to applied ethanol and its negative interaction with carbon dioxide were both increased.

The mean percentage concentrations of ethanol (w/w) in these plumules were as follows: carbon

Table 1. Ethanol Content (in μgm./5 Plumules) of 3-day-old Oat Seedlings, after Treatment for 3 days in Darkness with ± Carbon Dioxide (5 per cent) combined with ± Ethanol (0·3 per cent)

Mean values of 10 replicates in μgm. Exp. 1, plants not exposed to light; exp. 2, plants exposed on the third day for measurement; the lengths of mesocotyl + coleoptile + leaf (in mm.) are quoted in italics

Treatment: Carbon dioxide	+	+	−	−
Ethanol	+	−	+	−
Exp. 1	6·74	9·05	15·56	13·73
Exp. 2	10·17	11·53	21·31	16·95
	122·4	*211·2*	*177·5*	*283·3*

Variant	Analyses of variance of ethanol content			Analysis of covariance (total length and ethanol content)	
	DF	Mean squares Exp. 1	Exp. 2	DF	Mean square Exp. 2
Carbon dioxide	1	455·6 ↓	685·6 ↓	1	0·9
Ethanol	1	0·6	22·5 ↑	1	138·8 ↑
Carbon dioxide × ethanol	1	42·5 ↓	81·8 ↓	1	137·8 ↓
Error	36	5·1	7·8	35	4·7

The arrows indicate the direction of the effect.
DF, degrees of freedom.

dioxide and ethanol, 0·0096; carbon dioxide, 0·0063; ethanol, 0·013; controls, 0·0066. Evidently, the ethanol concentration within those plants treated with it did not approach that supplied externally. Further, treatment with carbon dioxide depressed the *in vivo* accumulation of ethanol when this was also administered in the culture medium, although under these conditions the content was higher than in the controls, indicating that some entry had occurred. Finally, as the same concentration of ethanol was present in the controls and under carbon dioxide, this treatment neither promoted the *in vivo* synthesis of ethanol nor did it bring about the decomposition of that occurring naturally.

The operation of this negative interaction between carbon dioxide and ethanol becomes clearly manifest when higher concentrations of ethanol are used in this type of experiment. In Fig. 1 are shown graphically the mean lengths of the mesocotyls and coleoptiles of etiolated oat seedlings, from eight treatments of a 2^3 factorially arranged experiment employing ± 0·5 per cent ethanol and ± 5 per cent carbon dioxide, both applied for 3 days from the time of planting, combined with exposure to dim red light (about 1 ft. candle) for 5 min. on termination of treatment with carbon dioxide and ethanol, when all seedlings were transferred in darkness to tap-water. The measurements of the third-day samples estimated the lengths of the plant organs at the time of exposure to light, and the differences in growth during the subsequent 5 days, displayed in the graphs, were after-effects.

In darkness (Fig. 1*A*) carbon dioxide (squares) initially reduced growth of the mesocotyl but afterwards occasioned a promotion, while 0·5 per cent ethanol alone (triangles) severely inhibited growth both during and after treatment. When, however, carbon dioxide was also administered, the initial depression of the mesocotyl due to ethanol gave way to rapid growth so that, ultimately, a length was attained equal to that reached in carbon dioxide alone (diamonds). The effect of illumination can be assessed by comparing the controls in Fig. 1, *A*, *B* (solid and open circles respectively); after exposure some elongation occurred, but more growth was shown by the mesocotyls of those plants treated with carbon dioxide. Ethanol alone severely inhibited growth, and again, simultaneous treatment with carbon dioxide relieved the inhibition so that a length was reached equal to that in carbon dioxide alone.

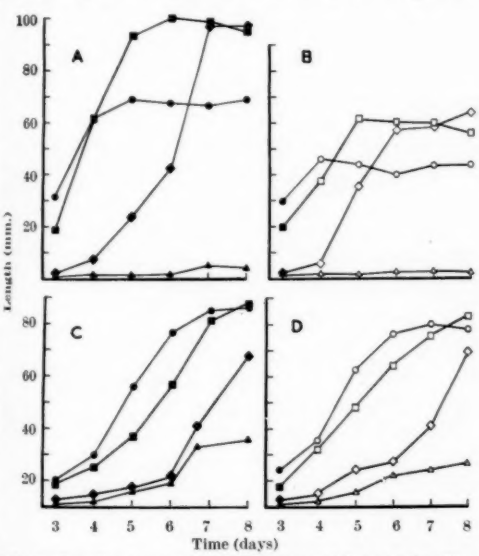

Fig. 1. Growth on successive days of mesocotyls and coleoptiles in darkness (*A* and *C*) and after a single exposure to dim red light for 5 min. on the third day (*B* and *D*) following treatment during the three days after planting, with: ◇ carbon dioxide + ethanol; □ carbon dioxide; *Δ* ethanol; ○ control
Solid points, in darkness; open points, after illumination

The behaviour of the coleoptiles is depicted in Fig. 1, *C*, *D* respectively, in darkness, and after exposure to light. The usual effects of light (transient promotion) and of carbon dioxide (generally depression) were recorded in this experiment. Ethanol alone inhibited growth, but again in the presence of carbon dioxide the inhibition was overcome; between the sixth and eighth days the rate of growth in darkness (1·02 mm./hr.) was approximately equal to that shown by the controls between the fourth and sixth days (0·98 mm./hr.). Similar effects were recorded for the plants exposed to light. Although the observations have not actually been made, the rates of elongation would suggest that the coleoptiles of the plants treated with carbon dioxide and ethanol together would ultimately have reached the lengths attained by their respective controls. The effect of this particular treatment may therefore be regarded as a delay in germination.

The inhibited seedlings (ethanol only) suffered no permanent injury; after transfer to water on the third day, root growth was resumed and in the last sample the second internodes were already quite long; the main apical meristem thus seems immune to externally applied ethanol.

The data as a whole suggest that ethanol has an inhibitory effect on growth which increases with the level of concentration. There is, as well, a secondary effect thought to arise from a conversion to carbon dioxide which then initiates a growth promotion. As these two effects are antagonistic the ultimate response observed will depend on the relation between these two factors. Thus, low concentrations of ethanol (0·2–0·3 per cent) are promotive whereas 0·5 per cent is inhibitory. In the first case the concentration in the tissues may not reach an inhibitory level and the promotive effect arising from its decomposition therefore appears. In the second case the inhibitory effect of ethanol masks the promotive effect of carbon dioxide.

This still leaves open the question as to why the inhibitory effect of 0·5 per cent ethanol can be eliminated by simultaneous treatment with carbon dioxide. Since both coleoptiles and mesocotyls behave in the same way some basic growth process seems to be affected by this dual treatment. The only data relevant are those in Table 1, which suggest that a high level of carbon dioxide prevents the *in vivo* accumulation of ethanol. Whether or not this is due to an activation of ethanol dehydrogenase by carbon dioxide is not at present known.

The variations in growth induced by these substances might indicate an effect on auxin metabolism, and this aspect of the problem is at present under investigation.

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LETTERS TO THE EDITORS

COSMOLOGY

The Steady Universe with Charge Excess

Recently, Lyttleton and Bondi[1] put forward the suggestion that the expansion of the Universe is caused by the absolute values of the charges of the proton and the electron not being equal, thereby giving a net charge to the hydrogen atom, and causing mutual repulsion in the universe, which to a first approximation may be considered as composed of a uniform distribution of hydrogen atoms. The analysis of Lyttleton and Bondi may be simplified by the use of the creation potential N suggested by Watson[2], and this simplification is the main purpose of this communication.

If there is creation, Maxwell's equations modify to

$$\nabla \times \mathbf{E} + \frac{\partial \mathbf{B}}{\partial t} = 0 \qquad \nabla . \mathbf{B} = 0 \qquad (1.1),(1.2)$$

$$\nabla \times \mathbf{H} - \frac{\partial \mathbf{D}}{\partial t} = \mathbf{J} - \frac{1}{\mu_0} \nabla N \qquad (1.3)$$

$$\nabla . \mathbf{D} = \rho + \varepsilon_0 \frac{\partial N}{\partial t} \qquad (1.4)$$

where the rate of creation of charge per unit volume is given by :

$$\nabla . \mathbf{J} + \frac{\partial \rho}{\partial t} = \frac{1}{\mu_0} \left\{ \nabla^2 N - \mu_0 \varepsilon_0 \frac{\partial^2 N}{\partial t^2} \right\}$$

where μ_0, ε_0 are the permeability and dielectric constant respectively of free space. (The Giorgi system of units is used throughout.)

Suppose now that a steady state is assumed. All derivatives with respect to t vanish and equations (1.1) to (1.5) become

$$\nabla \times \mathbf{E} = 0 \qquad \nabla . \mathbf{B} = 0 \qquad (1.6)\ (1.7)$$

$$\nabla \times \mathbf{H} = \mathbf{J} - \frac{1}{\mu_0} \nabla N \qquad (1.8)$$

$$\nabla . \mathbf{D} = \rho \qquad (1.9)$$

the rate of creation per unit volume being

$$\nabla . \mathbf{J} = \frac{1}{\mu_0} \nabla^2 N$$

A possible solution is clearly given by

$$\mathbf{E} = -\nabla V,\ \mathbf{H} = 0,\ \mu_0 \mathbf{J} = \nabla N,\ \rho = \varepsilon_0 \nabla^2 V \qquad (1.11)$$

and a solution of this type will be used in the next section. It will be noticed that, because of N, zero $\mathbf{H}$ is possible even when $\mathbf{J}$ is non-zero.

Suppose now that the charge on a proton is e and that the charge on an electron is $-(e - \Delta e)$. Let M be the mass of a hydrogen atom, its charge being Δe. Let n be the number of hydrogen atoms per unit volume (assumed uniform). The charge density is thus $n\Delta e = \rho_e$ and the mass density is $nM = \rho_m$.

Inside this, considered as a uniform spherical distribution the centre of which is the origin, the electric and gravitational intensities are respectively ($\mathbf{r}$ being the position vector from the origin)

$$\mathbf{E}_e = \frac{1}{3} \frac{\rho_e}{\varepsilon_0} \mathbf{r} \quad , \quad \mathbf{E}_m = -\frac{4\pi\gamma\rho_m}{3} \mathbf{r} \qquad (2.1),\ (2.2)$$

It will be observed that E_e satisfies equation (1.11) if

$$V = -\frac{1}{6} \frac{\rho_e}{\varepsilon_0} \mathbf{r}^2$$

The force on a hydrogen atom in this medium is, if $\mathbf{f}$ be the acceleration :

$$M\mathbf{f} = \Delta e \mathbf{E}_e + M\mathbf{E}_m = \frac{n}{3} \left[\frac{(\Delta e)^2}{\varepsilon_0} - 4\pi\gamma M^2 \right] \mathbf{r} \qquad (2.3$$

It follows from equation (2.3) that the sign of Δe is irrelevant. This point does not seem to have been mentioned by Lyttleton and Bondi, and so the theory applies equally well whether the charge of the electron or of the proton be the greatest in absolute value.

Using the relation between velocity and acceleration

$$\mathbf{f} = \frac{\mathrm{d}\mathbf{v}}{\mathrm{d}t} = \frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v}.\nabla)\mathbf{v} = (\mathbf{v}.\nabla)\mathbf{v} \qquad (2.4)$$

it follows that

$$\mathbf{v} = \left[\frac{n}{3M} \left(\frac{(\Delta e)^2}{\varepsilon_0} - 4\pi\gamma M^2 \right) \right]^{1/2} \mathbf{r} \qquad (2.5)$$

In order to obtain the proper behaviour at the origin, the arbitrary constant is put zero. This represents in the large a recession outwards with a velocity directly proportional to the distance. If the negative root were taken there would be an approach instead of a recession.

$$\mathbf{v} = \frac{\mathbf{r}}{\tau} \qquad (2.6)$$

where τ is the Hubble time.

The current flow vector associated with the field distribution discussed in section 2 is :

$$\mathbf{J} = \rho_e \mathbf{v} = n\Delta e \mathbf{r}/\tau \qquad (3.1)$$

Thus the rate of charge creation per unit volume is

$$\nabla . \mathbf{J} = \frac{3n\Delta e}{\tau} \qquad (3.2)$$

This is equivalent to a rate of production of $3n/\tau$ hydrogen atoms per unit volume in agreement with Lyttleton and Bondi. Were the negative root taken in (2.5), there would be annihilation instead of creation of matter.

One further consequence of experimental data may be noted, as it does not seem to have been referred to by Lyttleton and Bondi. There is experimental reason to believe that[3]

$$\frac{8}{3}\pi\gamma\rho_m\tau^2 = U^{-1} \qquad (4.1)$$

where U is a number the value of which is about unity. It follows, therefore, that

$$U^{-1} = \frac{8}{3}\pi\gamma n M \left[\frac{n}{3M} \left(\frac{(\Delta e)^2}{\varepsilon_0} - 4\pi\gamma M^2 \right) \right]^{-1} \qquad (4.2)$$

whence

$$\frac{(\Delta e)^2}{4\pi\varepsilon_0} = \gamma M^2 (2 + U) \qquad (4.3)$$

This implies that the electrical repulsion between two hydrogen atoms must be at least twice the

gravitational attraction. Were U unity, it would be 3 times, and were the repulsion 5 times the attraction, as suggested by Lyttleton and Bondi, this would imply that $U = 3$.

I am indebted to Dr. R. A. Newing for discussions.

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PHYSICS

Topology of Linear Neutron Kinetics

In the theory and interpretation of pulsed neutron measurements and in the analysis of random fluctuations in a nuclear fission reactor it is often helpful to study the kinetic behaviour of the time-dependent, space-independent model of the multiplying assembly.

For this purpose the neutron kinetics may be formulated as an integral equation as opposed to the more conventional set of first-order differential equations. This has some advantages in that : (i) The kernel functions of the Volterra-type integral equation have immediate physical significance. (ii) The integral equation is logically related to the topological representation of the fission process. It is also related in structure to the generating functions of probability theory. (iii) Numerical computation of resolvent kernels is usually more efficient than forward integration. (iv) Extension of the theory to non-stationary reactor kinetics and non-linear reactor topology is obvious.

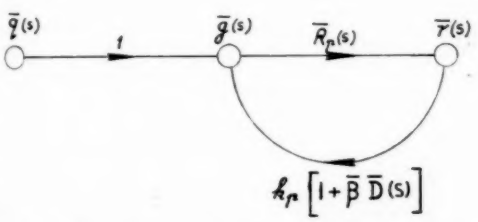

Fig. 1. k_p, Effective prompt multiplication constant; $\bar{q}(s)$, extraneous neutron source; $\bar{g}(s)$, mean generation rate of total importance; $\bar{r}(s)$, mean prompt regeneration rate of total importance; $\bar{R}_p(s)$, prompt importance regeneration kernel; $\bar{\beta}$, delayed neutron fraction; $\bar{D}(s)$, delay distribution function

The role of a nuclear reactor as a 'neutron amplifier' has been shown by Schmid[1]. His signal flow graph for linear topology in the frequency domain is given in Fig. 1. The integral equation of neutron kinetics then takes the form :

$$\frac{r(t)}{r_0} = 1 + \int_0^t G(t-\tau)\rho(\tau)\frac{r(\tau)}{r_0}\mathrm{d}\tau \qquad (1)$$

where $G(t)$ is Green's function of neutron kinetics and, for the case of stationary kinetics, obeys the following relationship :

$$G(t) = L^{-1}\left[\frac{1}{s\{F(s)+\tau\}}\right] \qquad (2)$$

where

$$F(s) = \frac{1}{s}\left[1 - D(s)\right] \qquad (3)$$

and

$$\tau = \frac{l}{\bar{\beta}k_p} \qquad (4)$$

and

$$\rho = \frac{k_p - 1}{\bar{\beta}k_p} - 1 \qquad (5)$$

l being the mean neutron generation time.

On the basis of this theory, the computation of reactor frequency and transient responses is carried out completely automatically on a digital computer. Step, pulse, ramp, sine and arbitrary time-responses can be studied for fixed, circulating fuel and boiling-water reactors.

In Fig. 2 some solutions of the integral equation are given for linear variations in reactivity. The frequency responses of a boiling water reactor are given in Fig. 3 and Fig. 4.

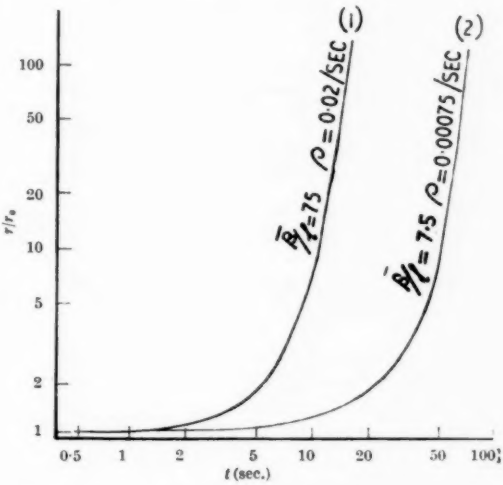

Fig. 2. Solutions of integral equation. (1) Water-moderated research reactor; (2) graphite-moderated power reactor

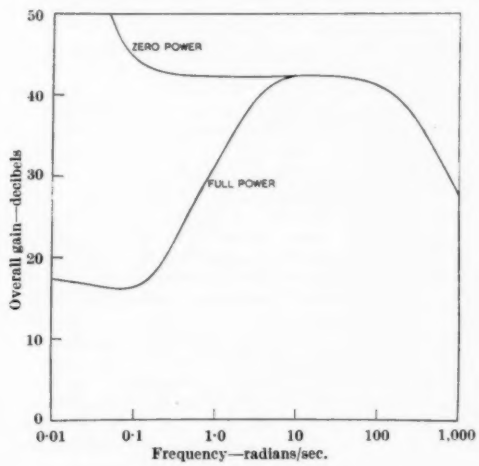

Fig. 3. Overall reactor transfer function

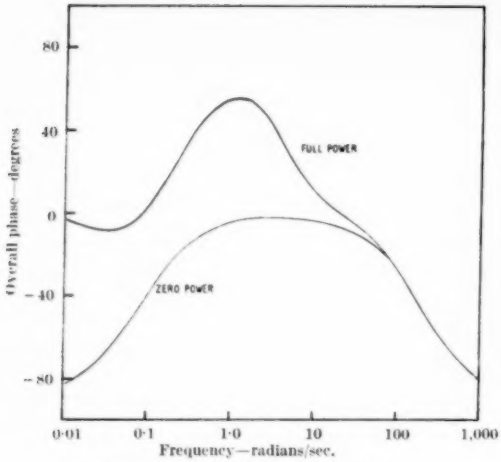

Fig. 4. Overall reactor transfer function

A full report will be published elsewhere.

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Molecular Emission Spectroscopy from 2μ to 12μ by a Michelson Interferometer

The combination of a two-beam interferometer and Fourier transformation has a great advantage over conventional spectrometers in the effective use of available energy[1,2]. Further, when the interferometer is of a type which has circular symmetry like Michelson's, there is another gain[3], giving the Michelson–Fourier combination an effective luminosity that is the highest of all known spectroscopic systems[4]. It is most advantageously used in wave-length regions where the limit to the spectroscopic resolution obtainable in a given time of observation is set by detector noise. So in the infra-red region, considerable improvement in spectra over those given by conventional methods is to be expected. This has been realized in practice in the very near infra-red[5] and in the far infra-red[6,7]. This communication describes its application in the 2–12μ region, where many molecular vibrational bands are found.

To test the system, experiments have been made to obtain emission spectra of gases at relatively low temperature in cases where the spectra are well known from absorption measurements. The gas was contained in a heated cell with rock salt windows and, after chopping, the radiation passed through a Michelson interferometer to a detector, as shown diagrammatically in Fig. 1. The interferometer was designed and constructed for this work by the Light Division, National Physical Laboratory. It has, as special features, an optically worked glass slide to constrain the moving mirror, and disks of barium fluoride 10 cm. in diameter for the beam splitter and compensator plates, but details of this instrument will be the subject of a separate communication. Measurement of the displacement of the moving mirror to get path-difference was made by illuminating part of the interferometer field with mercury (λ = 1·014μ) radiation and recording fringes by a photomultiplier. The interferogram of the radiation of the emitting gas was sampled at equal intervals of path-difference by a signal derived from the record of the mercury fringes. The values of intensity at these points were digitized and punched on paper tape suitable for input to the digital computer *Ace*, where a programme for Fourier transformation gave the spectrum.

Fig. 2 shows part of the ν_3 band of ammonia obtained in this way. For this spectrum, the gas temperature was 70° C., the pressure was 11 mm. mercury and the detector was a Unicam Golay cell fitted with an indium antimonide filter. The maximum path-difference introduced was 2 cm., this limit being imposed by the fall-off in visibility of the 1·014μ mercury fringes. This is attributable to the rather

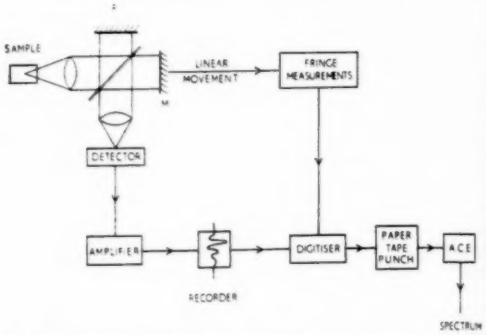

Fig. 1. Diagram of the arrangement of a Michelson interferometer used for emission experiments where the interferogram is digitized and recorded in a form for computer input

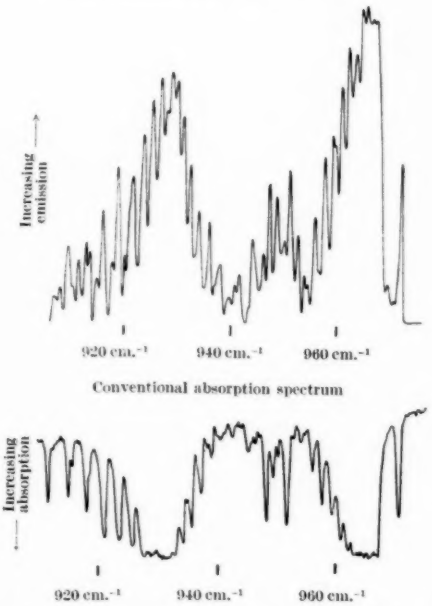

Fig. 2. Spectrum of part of the ν_3 band of ammonia in emission. The gas temperature was 70° C. and its pressure 11 mm. mercury in a cell of 10-cm. length. Below is shown a conventional absorption spectrum

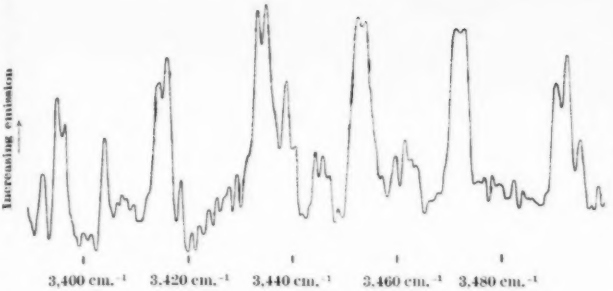

Fig. 3. Part of a spectrum near 3μ of ammonia in emission. The gas temperature was 75° C. and the pressure 600 mm. mercury in a 10-cm. cell

high pressure in the lamp used (Osram type *MBD* 125 W.). Following the convention which was adopted in far infra-red work[6], we define the resolution to be the reciprocal of the maximum path-difference, giving in this case a value of 0·5 cm.$^{-1}$. This is a restrictive definition, as may be seen by comparing the actual resolution of the emission spectrum with the conventional absorption spectrum shown below it. The latter was chosen from the literature[8] as most closely matching our resolution and pressure conditions. The resolution claimed for it is 0·4 cm.$^{-1}$, and it can be seen that the resolution in the emission spectrum is slightly better. When a comparison is made with the recent measurements of this band of ammonia at higher resolution by Mould, Price and Wilkinson, it is found that our resolution approaches their value of 0·1 cm.$^{-1}$, but the much lower gas pressure used in that work makes an exact comparison difficult.

The emission interferogram was recorded in about 1 hr. and the time taken by the *Ace* computer to calculate the part of the spectrum shown was about 15 min. The output of the computer is plotted automatically on a recorder, and the figures shown are direct reproductions of these plots.

Comparison with an emission spectrum made with a prism spectrometer[9] shows that much superior resolution and signal-to-noise ratio is possible with the interferometer in comparable times of observation.

Fig. 3 shows a small part of the spectrum of ammonia in emission near 3μ. Here the gas was at a temperature of 75° C., the pressure was 600 mm. mercury in a 10-cm. cell and the detector used was a lead sulphide cell with a germanium filter. The maximum path-difference was about 1·6 cm., giving a resolution of 0·6 cm.$^{-1}$. In this case it has not been possible to provide a suitable absorption spectrum for comparison, because in this region[10] three bands, ν_3, ν_1 and $2\nu_4$, all overlap and intensity distributions vary greatly. However, one expected feature, namely, the inversion splitting[11], can be seen in all the strong lines, despite the rather high gas pressure that was used.

From these results it can be seen that the Michelson–Fourier combination offers new prospects for the emission spectroscopy of sources of low brightness. The instrument described here is now being used for work on night-sky emission and also for observations on planetary atmospheres. Another possibility being investigated is the application to Raman spectroscopy with excitation lines of longer wave-length than those normally used. Finally, it is thought that advantages of this system, giving as it does the maximum product of solid angle and resolution, may be of importance in applications to artificial satellites, where the time-efficiency of the initial measurement of the radiation outweighs the disadvantage of having to compute the spectrum.

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Pressure Differences in a Direct-Current Capillary Discharge

THIS communication deals with the difference of pressure which arises between the ends of the capillary of a d.c.-excited krypton discharge lamp as a result of the motion of ions and electrons in the electric fields[1] of the discharge. The effect is of importance in view of the adoption of the 0·606μ line emitted by krypton-86 as the new standard of length[2]. The wave-length of this line can be shifted, because of broadening due to pressure, by an amount which depends on the pressure and the current in the discharge; this shift is of the order of one part in 10^8 under the conditions for excitation by means of a d.c. discharge, which have been recommended by the International Committee of Weights and Measures[3].

The measurements were made by means of a special lamp, which is illustrated schematically in Fig. 1. The form of this lamp was basically the same as that recommended for excitation of the primary wave-length standard, having a capillary of 3 mm. internal diameter and 90 mm. length. However, instead of a single hot cathode, two were provided, one at each end of the capillary, so that the d.c. discharge could be run in either direction. A Pirani gauge head was connected to each end of the capillary, for the measurement of the pressure, and a side-tube connected to one of the ends enabled control of the vapour pressure of the krypton by the use of a

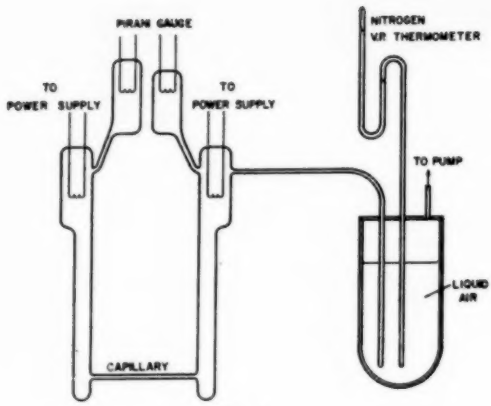

Fig. 1

cryostat. A nitrogen vapour-pressure thermometer was used to measure the temperature of the cryostat, and the resultant vapour pressure of krypton in the side-tube was deduced from the Meihuizen formula[4]. Although the pressure at one end of the capillary was controlled by the vapour pressure of krypton in the side-tube, which was held at temperatures down to 61° K., the temperature of the capillary itself was controlled by immersion in nitrogen at its normal boiling point.

The results of the measurements for a range of pressures and currents are shown in Fig. 2. The large relative differences of pressure between the two ends of the capillary are quite striking. Of particular interest in connexion with the primary wave-length standard is the pressure difference under the operating conditions as now recommended, namely, a current density of 3·0 m.amp./mm.^{2} with the krypton pressure controlled by its vapour pressure at the anode end, the temperature of which is at the nitrogen triple point. In this case the pressure is 32μ at the anode end and 21μ mercury at the cathode. According to typical values given for the shift due to pressure of the 0·606μ line of krypton-86 (ref. 5), the wave-length emitted by the gas in the discharge will vary along the length of the capillary by about 2·5 parts in 10^9. When the lamp is observed end on, as recommended at present, the possible error in the wave-length is not large compared with the accuracy specified by the International Committee (< 1 in 10^8), but it is clear that the effect must be taken into account if much greater accuracy is attempted with this form of source.

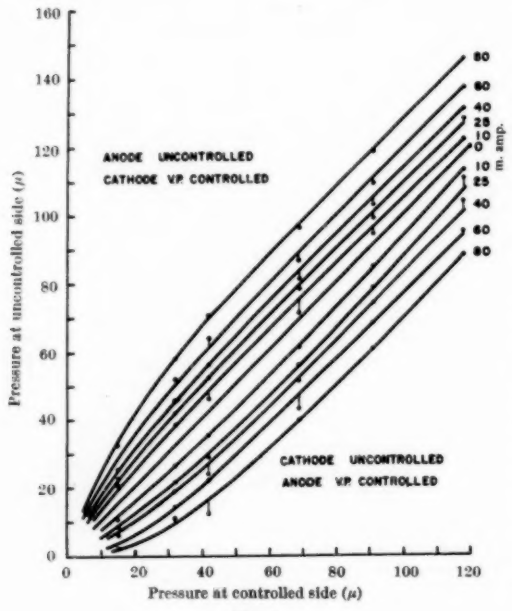

Fig. 2

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METEOROLOGY

Radar Reflectivities of Hail Samples

In the past few years quantitative measurements have been made of the radar reflectivity of many storms, particularly severe ones in which hail and even tornadoes have occurred. In particular, Donaldson has published and discussed vertical profiles of reflectivity of storms in the eastern United States[1]. He finds that the absolute magnitude of the reflectivity increases with the severity of the thunderstorm in the sequence rain, hail, large hail and tornadoes; and that in hail and tornado situations, the maximum reflectivity is found aloft at a height which increases with storm severity.

The proper interpretation of such observations clearly depends on an understanding of the characteristics (as radar targets) of hail, both wet and dry. Recent computations by Herman and Battan[2] and measurements by Atlas *et al.*[3] have shed light on the problem of radar scatter by hail (particularly large hail); and Atlas and Ludlam[4] have calculated the reflectivities of hail of various size-spectra. The question arises as to exactly what size-spectra do in fact exist, and the reflectivities which can be expected from such spectra.

Power returned to the radar is proportional to the reflectivity of the target, that is, to the sum of the back-scattering cross-sections. For spherical target elements of diameter much less than the radar wave-length (Rayleigh scattering) the back-scattering cross-section is proportional to the sixth power of the particle diameter, of which the sum (ΣD^6) is called Z, the reflectivity factor[5]. It has become customary to examine Z in relation to precipitation-rate and liquid water (or ice) content in the cases of rain and snow in which Rayleigh scattering applies. Where hailstorms are concerned it is useful in making comparisons to describe the reflectivity of a storm in terms of the equivalent reflectivity factor Z_e, that is, that value of ΣD^6 which, assuming Rayleigh scattering from spherical water drops, would yield the same reflectivity as is in fact observed.

In the course of a hailstorm-observing programme * in western Canada (Alberta), 28 samples of hail were collected. Each sample represents all the hail which fell on a small area (of the order of 0·05 m.²) during a hailstorm. The samples were collected from points over a wide area and on many different days; they represent a wide range of rates of hail-fall (0·02–40 mm. hr.⁻¹, melted) and of ice concentrations (0·01–6 gm. m.⁻³). For each sample, Z_e was computed on the basis of reflectivities derived by Atlas and Ludlam[4] for both wet and dry hail at radar wave-lengths of 3·3 and 10 cm. Each of the 28 values thus computed was plotted against the corresponding value of R, the rate of precipitation of the hail as observed at the ground.

Fig. 1 shows the plot of Z_e versus R, for both wet and dry hail at wave-length 3·3 cm. The straight lines show the least-squares regressions, expressed by the equations:

$$Z_e = 4 \times 10^4 R^{0.88} \text{ (wet hail)}$$
$$Z_e = 1 \times 10^4 R^{1.08} \text{ (dry hail)}$$

Z_e being in mm.⁶ m.⁻³ and R in mm. hr.⁻¹. Clearly, for the radar wave-length considered and for these particular hail distributions, wetting of the hail enhances Z_e by a factor of from 2 to 4. According to the recent reflectivity data mentioned above[2–4], such an enhancement is to be expected when the major contribution to the total reflectivity of the sample is made by hail of diameter less than about 1·5 cm. This is in fact the case in the Alberta samples where, in 21 of the 28 samples, 75 per cent of the total reflectivity at 3·3 cm. wave-length was contributed by hail of diameter less than 1·6 cm.

The regression lines are reproduced in Fig. 2 (*d* and *e*) with the corresponding ones for wave-length 10 cm. (*b* and *c*). Also shown (*a*) is the relation between Z ($= \Sigma D^6$) and R for the same 28 samples.

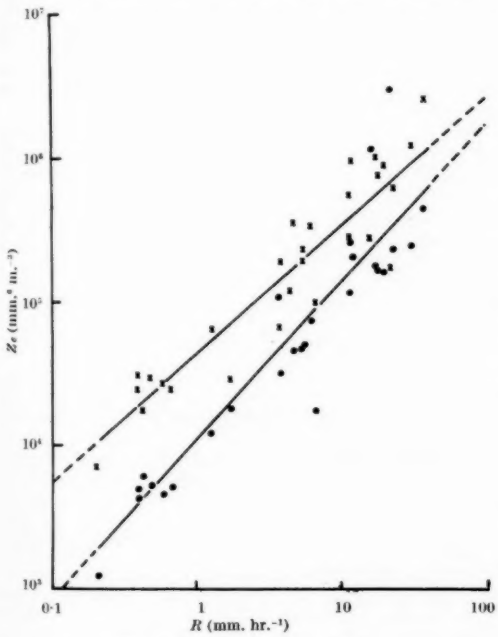

Fig. 1. ●, Dry hail; ×, wet hail

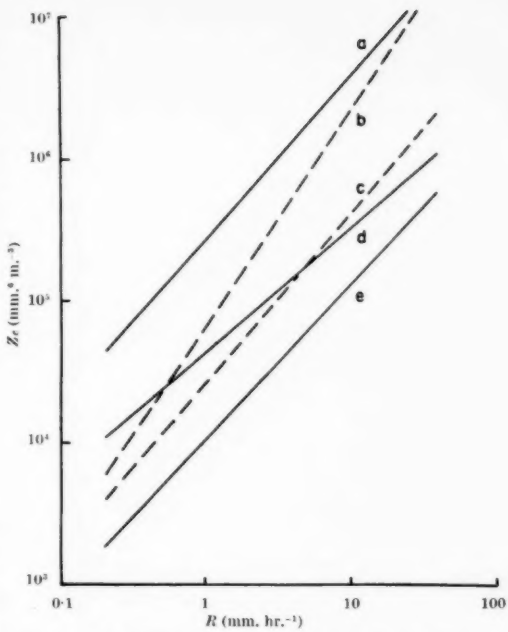

Fig. 2. *a*, $Z = \Sigma D^6$; *b*, Z_e wet 10 cm.; *c*, Z_e dry 10 cm.; *d*, Z_e wet 3·3 cm.; *e*, Z_e dry 3·3 cm.

This last relationship will clearly lead to an underestimate of hailfall-rate on the basis of power returned to the radar. As in the case of the 3·3 cm. wave-length, so also does wetting of the hail enhance the reflectivity at the longer wave-length, particularly at high precipitation-rates.

Table 1. EQUIVALENT REFLECTIVITY FACTOR Z_e, MM.⁶ M.⁻³

	LQ	*M*	*UQ*
Donaldson (ref. 1)	$1·5 \times 10^5$	10^6	4×10^6
Alberta (wet)	3×10^4	2×10^5	5×10^5
Alberta (dry)	8×10^3	6×10^4	2×10^5

Donaldson[1] has published vertical profiles of the median (M), the lower (LQ) and the upper quartile (UQ) values of Z_e for hailstorms measured by means of 3·3 cm. radar, and it is of interest to compare values taken from his curves at a low level (5,000 ft.) with the Alberta values for the same wave-length (see Table 1). The Alberta values are lower than Donaldson's by factors between 5 and 20. The agreement may very well be better in view of doubts as to the calibration of Donaldson's radar; in fact, a reduction of his values by a factor of 6 is suggested by Atlas and Ludlam[4].

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* Sponsored by the Alberta Research Council, the Canadian Meteorological Service and the National Research Council of Canada.

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METALLURGY

Hydrogen Reduction Characteristics of Natural and Artificial Hæmatites

Published data on the rate and mechanism of the reduction process in iron oxides are frequently discordant. This lack of agreement is exemplified by two recent studies[1,2]. McKewan[1], for example, has reported the rate of reduction of hæmatite spheres in the size-range 0·6–2·5 cm. to increase continuously from 400 to 1,050° C. I found[2], however, that hæmatite particles in the size range 0·002–0·008 cm. showed a rate maximum at about 550° C. and a pronounced rate minimum at 700° C. approximately.

Since this difference in behaviour could be due to a particle-size effect, an apparatus-effect or to different physical properties of the materials, it was decided to repeat the work eliminating the first two of these variables, and using hæmatites similar to those employed in the two investigations in question[1,2].

For one series, approximately spherical particles of artificial hæmatite, 0·03 cm. diameter, were prepared by rolling pigment grade ferric oxide (as used by McKewan) in a jar for 1 hr. A screened fraction of this material was fired, in a platinum boat, for 2 hr. in an atmosphere of oxygen at 1,370° C. and then cooled slowly in oxygen to room temperature.

For the other parallel experiments, particles similar in size and shape to the artificial hæmatite were obtained by screening a washed natural hæmatite from Yampi Sound, Western Australia. Part of this material was subjected to the same treatment as the artificial hæmatite and another portion was dried in air at 110° C.

Particles of both artificial and natural hæmatite, approximately 0·003 cm. diameter, were also prepared from screened fractions of the raw materials and dried in air at 110° C. Using a procedure described in ref. 2, 100-mgm. samples of the various materials were reduced in a stream of hydrogen flowing at 2,000 ml./min. The distinctly different results given by the natural and artificial hæmatites are shown in Fig. 1.

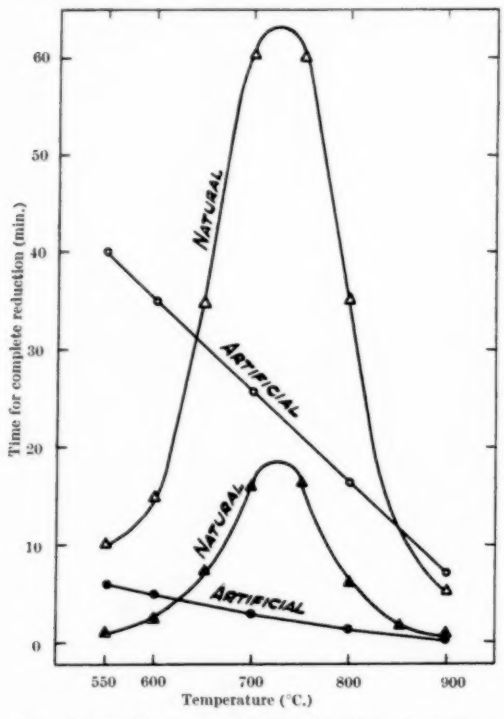

Fig. 1. Dependence on temperature of hydrogen reduction-rate for natural and artificial hæmatites. Δ, 0·03 cm. natural hæmatite, fired at 1,370° C. or dried at 110° C.; ▲, 0·003 cm. natural hæmatite dried at 110° C.; ○, 0·03 cm. artificial hæmatite fired at 1,370° C.; ●, 0·003 cm. artificial hæmatite dried at 110° C.

In Table 1 some physical properties of the two hæmatites are shown. Both materials are weakly magnetic, and heating them to 500°, 700° and 900° C. respectively, in nitrogen, and quenching, did not alter significantly the lattice parameters shown in Table 1. The only significant differences found so far in the two hæmatites are that, under reflected polarized light, the artificial material shows a pronounced 'sub-structure', while the natural hæmatite shows none and the small amount of gangue material (mainly silica and alumina minerals) present in the natural hæmatite occurs as separate particles, whereas in the artificial material it is present with ferric oxide as composite particles.

Table 1. Physical Properties of Hæmatites

Property	Hæmatite	
	Natural	Artificial
Fe_2O_3 content (weight per cent)	99·1	99·5
Density (gm./cm.³)	5·20	5·10
Hexagonal parameters (Å.) a	5·035	5·033
c	13·75	13·75
Crystallinity *	Monocrystalline	Polycrystalline

* Reflected polarized light examination.

It has been clearly demonstrated that the origin of a hæmatite may influence markedly the dependence on temperature of its hydrogen reduction-rate. Until the reasons for this differing behaviour are discovered, it is apparent that care must be exercised in interpreting or comparing reduction data for hæmatites from different sources.

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ENGINEERING

Power Requirements and Scale-up in Flotation

As part of a systematic programme for correlating mineral engineering process variables[1,2], flotation machines are now being investigated. There is no need to stress the engineering importance of the flotation process. First applied more than fifty years ago for the recovery of fine sulphide minerals from Australian gravity plant tailings, to-day it is the

only method for treating the major base metal sulphide ores, and is becoming of increasing importance for non-metallic ores and coal. As a mineral engineering operation, it is often second only to fine grinding in power consumption. Despite this, empiricism largely prevails from choice of equipment to control of process variables.

Relationships between cell capacity, energy input, and flotation-rate have been suggested[3,4], and there are indications that cell power intensity and capacity are related[5]. However, the function of power in the flotation cell should not be misinterpreted. Apparatus of this type are essentially dissipative mechanisms in which the energy input ultimately appears as heat; any useful function (in this case, a separation of minerals) is incidental. Similar behaviour has been pointed out in connexion with ball milling[1], where the power is utilized in rotating an eccentric load, a negligible portion going into comminution. However, the power dissipation-level is responsible for the particular fluid-flow regime maintained within the cell, and this in turn is related to phenomena leading to flotation such as gas dispersion, particle suspension, and bubble-particle aggregation. Hence, the energy dissipation-rate which is related to the power requirements of the flotation impeller should be useful in correlating flotation cell performance with design factors.

For the present, flotation performance will not be considered beyond expressing the view that, once the power requirements can be related to the geometry and other mechanical features of cells, performance can then be related to the appropriate power number or index. The problem to be considered thus resembles the more familiar one of mixer evaluation in the chemical processing field, where correlations, using Power, Reynolds, Froude and Weber numbers, are well known[6-11]. In the following, the flotation cell will be treated as a baffled mixer; hence only the power number and the Reynolds number will be considered. To account for the effect of entrained air and suspended solids, air volume and pulp specific gravity are introduced, and the Power and Reynolds numbers are then defined respectively by:

$$N_p = \frac{Pg_c}{\rho N^3 d^5} \cdot \frac{1}{s(1-a)} \qquad (1)$$

$$N_{Re} = \frac{\rho N d^2}{\eta_l} \cdot \frac{s(1-a)}{f_1 f_2} \qquad (2)$$

Where P is net power (consumed power less transmission losses), g_c is the Newton's law conversion factor, d is impeller diameter, N is impeller rotational speed, ρ is density of fluid, η_l is viscosity of pure fluid, a is aeration volume, s is non-aerated pulp specific gravity, f_1 is a function of non-aerated pulp variables (ratio: non-aerated pulp viscosity/pure fluid viscosity), f_2 is a function of aerated pulp variables (ratio: aerated pulp viscosity/non-aerated pulp viscosity). f_1 and f_2 are still to be determined, though some aspects of the former have been investigated[12].

It is useful in equation (1) to replace d^3 by the cell volume, V, and to substitute power intensity Π for the term Pg_c/V. Also it will be noted that Nd is proportional to the linear peripheral speed πNd. So modified, equations (1) and (2) become:

$$N_p' = \frac{\Pi d}{\rho (Nd)^3} \cdot \frac{1}{s(1-a)} \qquad (1a)$$

$$N_{Re} = \frac{\rho (Nd) d}{\eta_l} \cdot \frac{s(1-a)}{f_1 f_2} \qquad (2a)$$

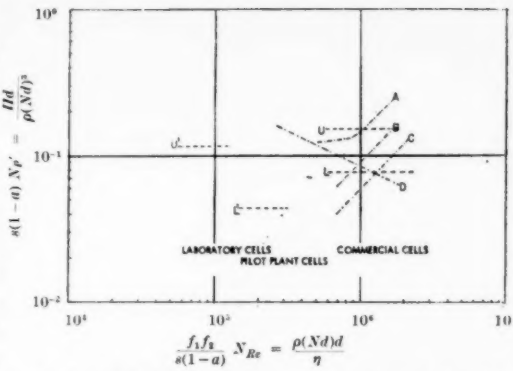

Fig. 1. Power factor – Reynolds number correlation for flotation cells

The literature is notably deficient in data for relating these dimensionless numbers. Without regard to the complex functions f_1 and f_2, there is little available on air entrainment volumes, and power figures where given at all usually are installed, rather than net values.

To make maximum use of the small amount of available data, attention was confined to Π, N and d. The constants for the fluid phase were ρ and η_l for water. With regard to the latter, Perry[6] suggests using the viscosity of the fluid phase when dealing with dispersions of gases in liquids; this is the procedure adopted here. Thus the groups become:

$$s(1-a)N_p' = \frac{\Pi d}{\rho (Nd)^3} \qquad (3)$$

$$\frac{f_1 f_2}{s(1-a)} N_{Re} = \frac{\rho (Nd) d}{\eta_l} \qquad (4)$$

Lines A, B, C and D (Fig. 1) are from approximate data supplied by four manufacturers. As in mixing, the radically different impeller design probably accounts for much of the difference in the relative positions of lines A, B and C.

The slope of unity for lines A, B and part of C follows from the scale-up criteria adopted, which (*a*) maintains constant peripheral speed for all cell sizes, and (*b*) estimates installed power from $\Pi \simeq$ constant. It will be noted from equations (3) and (4) that this is equivalent to plotting d against d. Line D is evidently based on different criteria. It is interesting to note that in baffled mixers N_p is almost independent of N_{Re} for $N_{Re} > 10^4$. Thus, in the absence of adequate mixer scale-up information (*b*) above is assumed, the value of Π being obtained from laboratory measurements, and for high Reynolds numbers N is calculated from $N_p \simeq$ constant[6,7,9].

Published and unpublished information for a range of commercial cells give values in the area enclosed by lines U and L. Estimated consumed power was used, and it is expected that net power values would be about 10 per cent lower. On the other hand, lines U' and L' enclose the area containing our own measurements on laboratory cells modelled after commercial cells, and here net power was used. Transmission losses for laboratory cells greatly exceed net power, and the use of consumed power would increase N_p about 10 times.

Thus, there is no reliable scale-up information between laboratory and commercial cells, or even for commercial cells alone.

XUM

In particular, the variation of peripheral speed and power intensity with cell size needs resolving. Once the relationships become established, they will provide a frame of reference for investigating the remaining operating variables. This should give an insight, for example, into the factors responsible for differences in the flotation-rate of the same material treated in different cells, both with regard to the size of cells and their design. In passing, it may be noted that this general correlation method should be applicable to the conditioning operation, which is likely to present fewer problems.

This possible correlation is brought to attention in the hope that adequate commercial data with which to establish this or some other correlation will now become available.

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CHEMISTRY

Nomenclature of Silica

For many years the term 'fused quartz' has been used for describing vitreous silica prepared by the fusion of crushed quartz crystals. In some quarters the term 'vitreous silica' has been reserved for material made from an acid-washed sand, usually containing larger amounts of impurities. Unfortunately, the term 'fused quartz' is often abbreviated to 'quartz' in the later pages of a publication, and sometimes even in the title. A quick glance at *Chemical Abstracts* subject index for 1956 revealed four abstracts dealing with "quartz", where it was obvious that vitreous silica was meant[1]. The electrophoresis of "quartz particles in a quartz cell" is mentioned in one abstract. It is highly unlikely that the electrophoresis cell was of crystalline quartz; it was presumably vitreous silica. The question remains, were the particles also of vitreous silica? A recent example of the misnaming of silica is a paper dealing with vitreous silica and probably cristobalite[2] and headed "Whisker growth from quartz". It contains the sentence "The quartz substrate . . . had a skin of devitrified quartz . . . ".

The term 'amorphous silica' is not used in any more consistent fashion. In general, it denotes an artificial silica filler or a naturally occurring material such as opal or diatomite. The artificial silica powder, of submicroscopic particle size and correspondingly high specific surface, may have been prepared by one of two types of process. A material made from an aqueous soil by drying will readily take up moisture; the particles will be porous. Silica powders prepared by a condensation of SiO_2 or SiO from the vapour phase will be less hygroscopic. A material such as 'Fransil', consisting of tiny spheres of silica glass, might be better classified as a fine vitreous silica. Diatomite is comparatively coarse, but extremely porous. If it has been calcined, it will have been partly converted to cristobalite or even tridymite, both toxic materials.

The chemical and biological properties of the various forms of silica depend markedly on the polymorphic form (vitreous silica, cristobalite, tridymite, quartz, coesite), particle size, porosity, and thermal history of the surface, even when the material is very pure. The examples cited show that there is a need for greater care and detail in the description of various forms of silica by means of such terms.

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Determination of Hydrogen in Gaseous Mixtures by Gas-Chromatography

In the analysis of gaseous mixtures by gas-solid chromatography using hot-wire or thermistor detectors, it is usual to reduce the signal to the recorder, when necessary, by an attenuating system in the form of a potentiometric chain so that only a known proportion of the signal is applied to the recorder. This method results in the main components of a mixture being measured less accurately than impurities or components of small concentration. This is particularly true when hydrogen is a main component, as it has a thermal conductivity seven or eight times as great as most other gases or vapours, necessitating a large measure of attenuation to keep its peak on the chromatographic chart.

The gaseous mixtures commonly analysed in this Laboratory contain about 40 per cent hydrogen, and the volume of gas necessary to achieve sensible peak measurements for the nitrogen present results in a hydrogen signal from a katharometer bridge of about 115 mV. In the past this signal has been attenuated to about 2·3 mV. to allow the peak to be measured on the 3-mV. potentiometric recorder used. Thus 40 per cent of hydrogen is represented by 2·3 mV. or a deflexion of 214·5 mm., and an error of 1 mm. in measurement leads to an inaccuracy of about 0·5 per cent in the bridge signal.

It was therefore decided to reduce the signal to the recorder by applying an accurately determined e.m.f. in opposition to the output from the katharometer bridge. The conventional apparatus used had recorder sensitivities of 3, 5, 15, 25, 50 and 150 mV. for full-scale deflexion. Of these the 15 mV. range was selected as being the most practical one to use. On this scale, for a gas containing 40 per cent hydrogen an effective back e.m.f. of 105 mV. is required to keep the signal to a reasonable position on the chromatographic chart. This is achieved by applying an e.m.f. of 21 mV. (that is, 105 × 3/15 mV.) across the potentiometric chain in opposition to the signal to the recorder. The recorder then registers a peak from a signal of 10 mV., giving a deflexion of 186·4

mm. Thus an error of 1 mm. in measurement leads to an inaccuracy of only 0·05 per cent of the bridge signal.

This device was demonstrated by applying fixed signals from the katharometer bridge and noting that for each mV. applied from the test-box the expected decrease in recorder response was obtained. The device was then incorporated into new chromatographic units under construction and has since been used in the analysis of more than 200 gaseous mixtures containing hydrogen as a major component.

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Heat Capacity and Resistivity Anomalies in Palladium Hydride

A recent paper on the electrical resistivity (ρ) of solutions of hydrogen in palladium[1] records maxima at 43° K. and 52° K. for the compositions (hydrogen/palladium) 0·60 and 0·54 respectively, whereas there is a linear fall of ρ when the hydrogen/palladium ratio is 0·48. A reproduction of the curves of ρ versus T by Schindler *et al.* is shown in Fig. 1. The maximum is evident in the graphs for the composition 0·60, but not for the composition 0·54; however, the authors state that for this composition there is a definite maximum in the points at the lower temperatures.

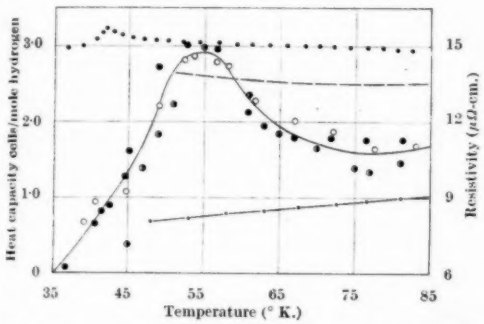

Fig. 1. Electrical resistivity and heat capacity per mole of hydrogen in certain solutions of hydrogen in palladium. △, Cp H/Pd = 0·50 (Nace and Aston); ○, Cp H/Pd = 0·25; ●, H/Pd = 0·125; ●●, ρH/Pd = 0·6; — —, ρH/Pd = 0·54; ●—●, ρH/Pd = 0·48

While the neutron diffraction work in ref. 2 leaves little doubt that there is hydrogen (hydrogen/palladium ~0·05) in the α-phase at room temperature, it is not unlikely that all the hydrogen is in the β-phase at lower temperatures.

Recent thermal studies in this laboratory have revealed slow exothermal processes on cooling solutions of hydrogen in polycrystalline palladium occurring near 150° K. and above 200° K. From the thermal drifts of the adiabatic calorimeter after various degrees of annealing, energies of activation of 6,400 and 2,900 calories were found for the upper and lower temperature processes respectively. In both cases graphs of the log of the rate of energy emission against $1/T$ gave good straight lines, except where annealing produced a state near equilibrium. This is in accord with anomalies in the heat capacity of hydrogen in palladium black (hydrogen/palladium = 0·50)[3] and one or both of these two processes may perhaps be thought of as connected with migration of hydrogens from the α- to the β-phase.

In view of the work on the resistivity[1] and of recent speculations concerning hysteresis in palladium hydride[4], heat capacity measurements for hydrogen in polycrystalline palladium with hydrogen/palladium ratios of 0·25 and 0·125 are of interest though perhaps not unexpected. The results, based on 1 mole of hydrogen in each solution, are given in Fig. 1 along with the earlier values for hydrogen/palladium equal to 0·50. The method used and the accuracy of the results are as given in ref. 3. Thus at hydrogen/palladium ratios of 0·125 and 0·25 the accuracy is respectively 2 per cent and 1 per cent of the heat capacity due to the hydrogen.

The heat-capacity maximum at 55° K. previously reported for a hydrogen/palladium ratio of 0·50 is not shifted at either composition and the results for both solutions lie on the same curve. The slow process occurring below 50° K. reported in ref. 3 was noted again. This is not connected with the two processes mentioned. By cooling the sample slowly through a temperature-range 30° above maximum, the slow process was 'annealed out' and all measurements were taken after this annealing.

The fact that the maximum is unchanged by composition at compositions of hydrogen/palladium below 0·50 might be taken to mean that all the hydrogen is in a phase of constant composition below room temperature. This would require migration of hydrogen from the α- to β-phase at low temperatures. However, if the hydrogen atoms are bound to palladium in such a way that there is little or no interaction between the protons, the heat capacities would not be affected very much by composition. This situation is unlikely. If the composition of the β-phase is constant, in view of the slow process occurring at the higher temperatures, it would seem that any process of ordering would most likely be one resulting in removal of some sort of rotational or librational process.

A comparison between the resistivity and heat-capacity data leaves little doubt that the maxima are connected. The results of Schindler *et al.*[1] indicate that the maximum in the resistivity curve may well be at 55° K. for a hydrogen/palladium ratio of 0·50. It is also not unlikely that the maximum in the heat-capacity curve would be shifted at higher concentrations of hydrogen. Heat capacities in this temperature-range at higher hydrogen concentrations will be studied.

Schindler *et al.*[1] suggest an anti-ferromagnetic transition as a possible cause of the maximum. The onset of rotational or re-orientation process used to explain the heat capacity maximum[3] would also produce an anomaly in $g(\nu)$ for the phonons and it is possible that the resistivity results might be explained on this basis. For compositions above Pd_2H it is not possible to connect the palladium atoms without bound hydrogen in endless chains[3].

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XUM

'Gel Filtration' of Organic Compounds

Following Porath and Flodin's description[1] in 1959 of gel filtration as a fractionation method, it has been regularly used in this laboratory for separation of proteins and polysaccharides from sugar cane juice and sugar factory syrups. Unpublished results of the Swedish workers suggested that the method might be extended to the fractionation of substances in a narrow range of quite low molecular weights. Because of the possibilities of the method materials other than cross-linked dextran have been investigated here as alternative media for gel filtration.

In our laboratory Mountfort has shown that 'Dowex 50' cation-exchange resin (Na^+ form) gives partial fractionation of a mixture of mono-, di- and trisaccharides in aqueous solution. Other workers have noted similar phenomena[2,3] and even a number of monosaccharides have been separated on 'Dowex 50' (Ba^{2+} form)[4]. As fractionation without the complications of ion exchange is often desirable, styrene-divinyl benzene copolymers, samples of which were obtained in bead form from the Dow Chemical Co., were tested. However, even with the least cross-linked resin, substances as widely different as egg albumin and glucose could not be separated in aqueous solution. Likewise, when alcohol was used as the liquid medium, no separation of such large and small organic molecules as eosin and formic acid could be achieved.

Meanwhile Vaughan[5] reported separation of polystyrene fractions in benzene on a cross-linked polystyrene which swelled to a very high degree in that solvent. He also suggested that fractionation of low molecular weight materials might be possible on less swollen resins, paralleling the separation of glucose from a dextran of number average molecular weight 1,000 by Porath and Flodin. We have now achieved the separation of several organic compounds using not only benzene but also a number of other liquids in which the resin swells to some extent.

The grades of copolymer available contained from 2–12 per cent divinyl benzene (DVB) and were designated *X*2, *X*4, *X*8 and *X*12. The swelling of each in a variety of solvents is shown in Table 1, being expressed as the ratio of swollen volume to dry volume (lightly packed).

Using columns of 1·4 cm. × 45–50 cm. and benzene as medium, almost complete separation of cholesterol and furfural was obtained with *X*2, *X*4 and *X*8 resins, but rather poor separation with the *X*12 resin. Cholesterol was eluted ahead of the furfural in each case.

Table 1. Swelling of Styrene–DVB Copolymers

Solvent	Swelling ratios (× 100) *X*2	*X*4	*X*8	*X*12
n-Butyl alcohol	105	—	—	—
iso-Butyl alcohol	105	—	—	—
sec.-Butyl alcohol	105	—	—	—
tert.-Butyl alcohol	105	—	—	—
n-Amyl alcohol	105	—	—	—
iso-Amyl alcohol	110	—	—	—
Pentan-1-ol	105	—	—	—
tert.-Amyl alcohol	105	—	—	—
Diethyl ether	190	180	140	135
Acetone	195	175	135	135
Methyl ethyl ketone	220	180	155	175
Ethyl acetate	235	210	160	140
Butyl acetate	260	210	125	130
Methylene chloride	370	250	235	190
Chloroform	380	285	230	175
Carbon tetrachloride	315	230	130	140
Dioxane	310	235	160	135
Benzene	400	280	210	170
Toluene	395	260	180	155
Petroleum ether (60–80°)	145	105	100	100
'Shell *X*2' (100°)	165	130	105	100

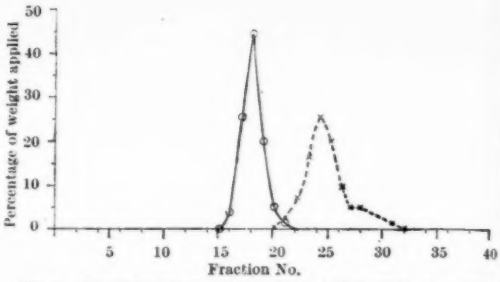

Fig. 1. Separation of resorcinol and 1-naphthylamine. ○, resorcinol; ×, 1-naphthylamine. Fraction volume, 5·1 ml.

With similar columns packed with *X*2 resin and using ethyl acetate or acetone, it was shown that it was possible to separate organic compounds with molecular weights in a much narrower range. Thus resorcinol and 1-naphthol (mol. wt. 110 and 144) when run separately in either solvent gave peak concentrations after different volumes of effluent and when run together were separated to a considerable extent. Resorcinol, although of smaller molecular weight, left the column ahead of the naphthol. Better separation of the two phenols was obtained in acetone than in ethyl acetate, although the resin swells less in acetone. By using a column 1·4 cm. × 140 cm. almost perfect separation of 0·4 gm. of each component was obtained.

To obtain quantitative data more readily, 1-naphthylamine was substituted for 1-naphthol. The phenol was determined by reaction with diazotized *p*-nitroaniline and the amine by its reaction with furfural in acetic acid. The separation of 0·1 gm. of each component in acetone solution is shown in Fig. 1.

Table 2. Effluent Volumes at Peak Concentrations

Substance	Molecular weight	Volume (ml.)
Acetaldehyde	44	165
Ethylamine	45	160
Ethanol	46	150
n-Pentanol	88	153
Furfural	96	167
Resorcinol	110	200
1-Naphthol	144	172
Alizarin	240	165
Quinalizarin	272	152
Cholesterol	387	126
Thymolphthalein	430	132

A number of compounds of varied structure were individually passed through a column 1·4 cm. × 135 cm. in benzene medium. The effluent volumes corresponding to peak concentrations of each were determined and are given in Table 2. The results suggest that gel filtration, as described by Porath and Flodin, is part of the mechanism involved in fractionation. However, the nature of each substance appears to be at least as important as its molecular weight. Thus, although cholesterol and thymolphthalein, both of high molecular weight, pass through the column ahead of the other compounds studied, a highly aromatic substance such as 1-naphthol will leave the column after aliphatic compounds of much lower molecular weight. In the aliphatic series the hydroxyl group causes relative exclusion (for example, compare ethanol and acetaldehyde). The extra hydroxyl group of resorcinol presumably explains its appearance ahead of 1-naphthol when these substances are run in acetone or ethyl acetate solution. In

benzene solution resorcinol behaves differently and leaves the column a considerable time after such compounds as 1-naphthol and furfural.

In this case and with certain other substances, adsorption may play some part. When the benzene-soluble base from methylene blue was run through *X2* resin, adsorption definitely appeared to occur, as there was a very large volume of effluent before the appearance of the dye, which was distributed in a much greater volume of solvent than usual.

The effect of the solvent is obviously great and the effect of the degree of cross-linkage of the resin is largely unknown. Much greater knowledge is required before a particular application or the optimum conditions can be predicted. However, the evidence suggests that here is a method which may assist in the isolation or purification of various natural or synthetic substances.

I am indebted to the Colonial Sugar Refining Co., Ltd., for permission to publish this communication.

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Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides

RECENTLY, Martin and Winter[1] suggested two new structures for trimeric titanium (IV) alkoxides. Their suggestion that $Ti_3(OR)_{12}$ may involve 6-co-ordinated titanium in a trigonal prismatic rather than an octahedral configuration is interesting but a decision on this must await the results of X-ray diffraction analysis. Besides providing a more symmetrical structure for the trimeric titanium chloride tributoxide they claimed that the trigonal prismatic structure also alleviates certain difficulties concerning the structures we proposed[2] for the polymeric titanium oxide ethoxides, which were based on the octahedral trimer structural unit. There are two points at issue. First, they consider on mechanistic grounds that the formation of the trilinear polymer species $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ "requires statistically unfavourable mutual orientation of two $Ti_3(OR)_8(OH)_4$ units before effective collision and expulsion of four water molecules could occur, etc.". Secondly, they mention that our structure for $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ involves an unknown 4-covalent bonding arrangement between oxygen and its four nearest neighbours. Concerning the first point it must be emphasized that our structure was developed to account for the variation of the number average degree of polymerization as a function of the degree of hydrolysis for a whole series of titanium oxide ethoxides. The mechanism of the hydrolysis was not considered, it is indeed unknown, and it is of no significance provided that a rapid rearrangement of the initial hydrolysis products occurs, thus resulting in the formation of the most stable structure. Moreover, we reported evidence which suggested that disproportionation takes place and this involves rapid structural rearrangements between members of the polymer series:

for example,

$$6\ Ti_9O_8(OEt)_{20} \rightarrow Ti_3(OEt)_{12} + 2\ Ti_6O_4(OEt)_{16} + Ti_9O_8(OEt)_{20} + Ti_{12}O_{12}(OEt)_{24} + Ti_{15}O_{20}(OEt)_{32}$$

An important feature of our structural series was the fact that the number average degree of polymerization is independent of the distribution of polymer sizes (as can be verified by inspection of the above equation) and is determined exclusively by the average degree of hydrolysis. The alternative structures for $Ti_3(OR)_{12}$ proposed by Martin and Winter will not account for the variation of the number average degree of polymerization with degree of hydrolysis even if the postulation that the titanium oxide ethoxides contain some terminal Ti=O groups were true. Thus although the formulation $(RO)^t_4(RO)^b_6Ti_3(O)^b_2Ti_3(OR)^b_6(O)^t_2$, where $(RO)^t$, $(RO)^b$, $(O)^t$ and $(O)^b$ are terminal alkoxide, bridging alkoxide, terminal Ti=O and bridging TiO groups respectively, will account for the compound $Ti_6O_4(OEt)_{16}$ the logical development of such a scheme would predict species which are not members of the $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ series, including a limiting infinite polymer $[Ti_3O_3(OEt)_6]_\infty$. We now report some infra-red spectra (in carbon disulphide solution) of $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$ in comparison with that of $Ti_3(OEt)_{12}$. Briefly stated, it appears that as the degree of hydrolysis is increased there is a decrease in intensity of the bands at or near 2,960, 2,900, 2,840, 2,680, 1,370, 1,350, 1,135, 1,102, 1,063, 1,038, 915 and 890 cm.$^{-1}$, which involve in various ways the ethoxide groups. Simultaneously there is an increase in intensity of bands at around 790 and 770 cm.$^{-1}$ which was absent in $Ti_3(OEt)_{12}$ and which must therefore be assignod to Ti·O·Ti systems. Hydroxyl bonds were not detected in these spectra, and this eliminates the possible presence of Ti—OH groups in the oxide ethoxides. According to Barraclough, Lewis and Nyholm[3] a strong band at 1,087 cm.$^{-1}$ was assigned to the Ti=O group in titanyl *bis*-acetyl acetonate while bands in the 800–900 cm.$^{-1}$ region in other 'titanyl' complexes were considered to be due to Ti·O·Ti·O·Ti· systems. Accordingly we conclude that the bands near 800 cm.$^{-1}$ in $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$ must be due to oxygen bridges between titanium atoms and that these compounds do not contain the Ti=O bonds proposed by Martin and Winter. It is noteworthy that Zeitler and Brown[4] considered that bands at 820 and 763 cm.$^{-1}$ in titanium oxide *n*-butoxide were due to Ti·O·Ti bonds. Thus, the trigonal prismatic alternative configuration for 6-co-ordinated titanium does not appear to have any supporting evidence.

The second objection to our structure, namely, the unfamiliar configuration of the quaternary oxygen, is more difficult to answer. The oxygen is situated at the apex of a tetragonal pyramid with titanium atoms in the basal positions. We tried several alternative structures in attempts to produce a tetrahedral co-ordination of the quaternary oxygens but without success. However, examples can be cited in which other first-row elements (for example, beryllium, boron) exhibit co-ordination numbers which are "not allowed" according to simple valency theory. Wheatley[5] has shown by X-ray diffraction on LiOMe that the oxygen is pyramidally bonded to four lithiums in this compound, and this provides a precedent for our Ti_4O configuration. There seem to be two alternative explanations of the 'pyramidal'

oxygen. First, if the four Ti—O bonds are covalent and equivalent then it is essential to invoke the participation of 3-centred and 5-centred molecular orbitals involving oxygen and two or four titaniums, respectively. The Ti_4O system would then involve only six of the eight valence electrons of the O^{2-}. The second possibility would require the Ti_4O group to be unsymmetrical with two Ti—O bonds being essentially covalent and considerably shorter than the other two, which would involve electrostatic attraction. It is relevant to note that the intermolecular bonds in $Ti_3(OEt)_{12}$ are fairly weak[6] while the infra-red-spectrum[7] does not appear to distinguish bridging ethoxide groups from terminal ethoxide groups. This could be taken as evidence that the intermolecular Ti—O bonds involve weak electrostatic binding. Preliminary results[8] on the high-resolution proton magnetic resonance of $Ti_3(OEt)_{12}$ in carbon disulphide at room temperature revealed only one type of ethoxide group. This suggests that the bridging and terminal ethoxide groups are exchanging positions extremely rapidly, and this reinforces our view that the hypothetical kinetic arguments of Martin and Winter are not relevant to the determination of the structures of titanium oxide ethoxides.

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The preceding communication by Bradley and Westlake is most interesting, for it provides new experimental data in the form of infra-red spectra and high-resolution proton magnetic resonance measurements on the polymeric titanium (IV) ethoxide and titanium (IV) oxide ethoxides.

Contrary to their above statement, however, one of the several logical developments of our scheme does, in fact, predict species which are members of the $Ti_{2(x+1)}O_{4x}(OEt)_{4(x+3)}$ series suggested by the workers at Birkbeck College, namely :

$$Ti_3(OR)_{12} + Ti_3(OR)_6(OH)_6 \rightarrow Ti_6O_4(OR)_{16} + 2ROH + 2H_2O$$

$$Ti_6O_4(OR)_{16} + Ti_3(OR)_6(OH)_6 \rightarrow Ti_9O_8(OR)_{20} + 2ROH + 2H_2O$$

$$Ti_9O_8(OR)_{20} + Ti_3(OR)_6(OH)_6 \rightarrow Ti_{12}O_{12}(OR)_{24} + 2ROH + 2H_2O$$

However, it differs significantly from their linear model in that the limiting polymer would be finite rather than infinite, with the formula $Ti_{12}O_{12}(OEt)_{24}$ rather than $[Ti_3O_4(OEt)_4]_\infty$ (ref. 1). It may be relevant that the painstaking investigations of the group working at Birkbeck College have led to the isolation and characterization of only two members of their $Ti_{2x+1}O_{4x}(OEt)_{4(x+3)}$ series, namely $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$, which suggests that these may be structures of special stability. No obvious points of stability are to be expected on their

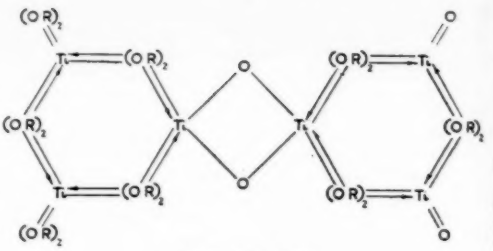

Fig. 1. $Ti_6O_4(OR)_{16}$

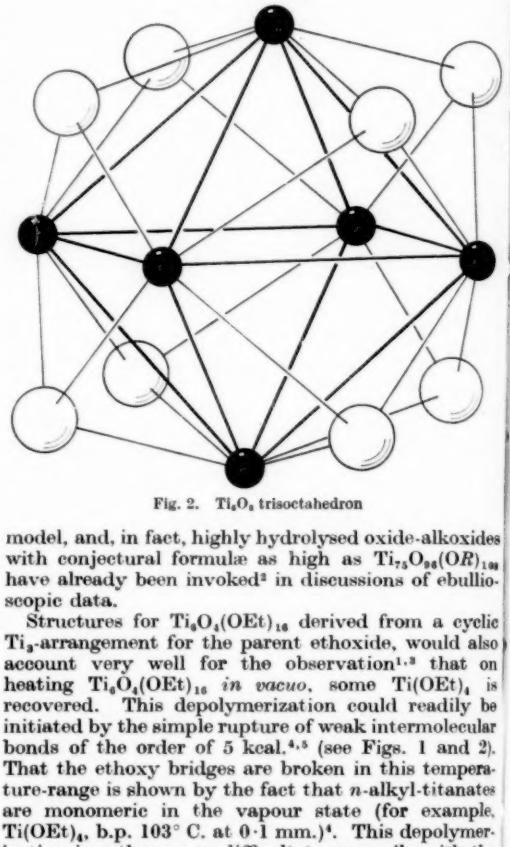

Fig. 2. Ti_6O_8 trisoctahedron

model, and, in fact, highly hydrolysed oxide-alkoxides with conjectural formulæ as high as $Ti_{75}O_{96}(OR)_{108}$ have already been invoked[2] in discussions of ebullioscopic data.

Structures for $Ti_6O_4(OEt)_{16}$ derived from a cyclic Ti_3-arrangement for the parent ethoxide, would also account very well for the observation[1,3] that on heating $Ti_6O_4(OEt)_{16}$ *in vacuo*, some $Ti(OEt)_4$ is recovered. This depolymerization could readily be initiated by the simple rupture of weak intermolecular bonds of the order of 5 kcal.[4,5] (see Figs. 1 and 2). That the ethoxy bridges are broken in this temperature-range is shown by the fact that *n*-alkyl-titanates are monomeric in the vapour state (for example, $Ti(OEt)_4$, b.p. 103° C. at 0·1 mm.)[4]. This depolymerization is rather more difficult to reconcile with the linear model for $Ti_6O_4(OR)_{16}$ (structure IV in our previous communication[6]), for each titanium atom is held in the structure by at least one strong covalent Ti—O—Ti bond, presumably of the order of 50–100 kcal. mole^{-1}, and a complete rearrangement would have to be postulated.

A further specific criticism made of our model is that it cannot accommodate the disproportionation scheme for oxide-alkoxide polymers. While we are not convinced of the occurrence of disproportionation in these compounds, Bradley and Westlake's criticism remains invalid, for numerical equations of the type illustrated here indicate that the number average degree of polymerization could also be independent of the distribution of polymer sizes for our model:

$$2[Ti_6O_4(OEt)_{16}] \rightarrow [Ti_3(OEt)_{12}] + [Ti_9O_8(OEt)_{20}]$$

$3[Ti_6O_4(OEt)_{16}] \rightarrow 2(Ti_3(OEt)_{12}] + [Ti_{12}O_{12}(OEt)_{24}]$

The case for disproportionation of $Ti_6O_4(OEt)_{16}$ was originally based on the time-independence of the number of osmotically active particles and the decrease in reactivity towards the Karl Fischer reagent which were observed on prolonged boiling in ethanol. Although Bradley, Gaze and Wardlaw[2] were unable to determine whether $Ti_6O_4(OEt)_{16}$ contained hydroxyl groups, they believed them to be absent, and therefore concluded that the reactive constituent must be bicovalent oxygen attached simply to titanium atoms, as in $\overset{\delta+}{Ti}—\overset{\delta-}{O}—\overset{\delta+}{Ti}$. The decrease with time in Karl Fischer reactivity was then ascribed to progressive replacement of bicovalent by tricovalent oxygen as the molecular complexity of the oxide-alkoxide increased. This provisional interpretation has not been revised in terms of their subsequent $Ti_{3x+1}O_{4x}(OEt)_{4x+3}$ model[1], and they have tacitly assumed that it continues to apply without modification.

It is not our purpose here to re-examine the case for disproportionation, but two comments will serve to illustrate that this thesis may be of doubtful validity. First, inspection of the linear model for $Ti_{3x+1}O_{4x}(OEt)_{4(x+3)}$ reveals that the number of bicovalent Ti—O—Ti groups (and of quaternary oxygen atoms) per titanium is, in fact, independent of molecular complexity, so that the decrease in reactivity to Karl Fischer reagent cannot be ascribed to this cause without either amending the original postulates or invoking long-range electronic effects. Secondly, the "apparent water content" of a crystalline sample of $Ti_6O_4(OEt)_{16}$ was found by them to decrease from 6·5 to 2·6 per cent simply by heating and recrystallizing the sample from ethanol (experiment *c*, ref. 3), so that the "active centres" cannot be the Ti—O—Ti groups as originally postulated.

With regard to the infra-red spectra reported by Bradley and Westlake, two points are at issue. First, we would agree with their conclusion that bands near 800 cm.$^{-1}$ in $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$ must be assigned to oxygen bridges between titanium atoms. We direct attention again, however, to the unfamiliar configuration of the quaternary oxygen involved in the linear model, and stress that no simple Ti·O·Ti·O·Ti system is involved here. Examination of the alternative linear and cyclic structures for $Ti_6O_4(OEt)_{16}$ reveals that bonding of the type $Ti\langle^{O}_{O}\rangle Ti$ occurs in both cases, and accordingly, their infra-red spectra in the 800 cm.$^{-1}$ region are unlikely to be dissimilar.

The second objection to our structure concerns the apparent absence of a Ti=O stretching frequency. Since the hydrolytic reaction scheme illustrated above represents only one of several possible schemes, the presence or absence of Ti=O groups in the hydrolysis products has little bearing on a decision between the linear and cyclic structures for the pure ester. For example, an attractive symmetrical close-packed $MoCl_4$-type structure can be envisaged for $Ti_6O_4(OEt)_{16}$ (Fig. 2), which does not involve terminal Ti=O groups. This structure, like our cyclic trimer, is again based on TiO_6-trigonal prisms with an octahedral skeleton of titanium atoms, with two terminal ethoxy groups attached to each. Each oxygen atom involved in the $Ti_6O_4(OEt)_4$-trisoctahedron is either 3- or 4-co-ordinated, in configurations, pyramidal or tetrahedral, both well established for oxygen.

In conclusion, we reiterate that the resolution of this polemic must ultimately await complete structure determinations of the ethoxide and two oxide-ethoxides, all of which can be obtained in crystalline form. Our original purpose remains, as stated previously, simply to direct attention to the fact that the linear model is by no means unique, and certainly, on the evidence available, does not yet enjoy the exclusive consideration claimed for it.

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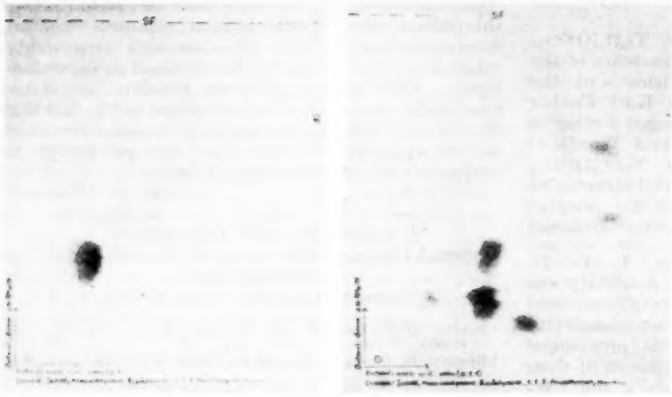
A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms

For chromatographic identification of iodide and iodinated protein derivatives, it is often necessary to locate inorganic and organic iodide tracers on the same chromatogram. In paper chromatography a number of methods have been used for the detection of iodide. These include spraying with silver nitrate[1]; potassium chlorate, acid and starch[2]; 1 per cent aqueous palladium chloride[3]; a mixture of ferric nitrate and hydrogen peroxide[4]; the ceric-arsenite reagent[5]; and a mixture of $FeCl_3.6H_2O$, $K_3Fe(CN)_6$ and $NaAsO_2$ (*FFCA* reaction)[6]. Most of these tests for iodide interfere with those commonly used for the identification of iodinated amino-acids, namely, ninhydrin[7], diazotized sulphanilic acid[8], or its diethyl amide[8]. The ceric-arsenite and the *FFCA* reagents are the most sensitive (0·01 μgm. I^-) and can moreover be used for the detection of inorganic iodide, together with iodinated amino-acids on the same chromatogram[6,9]. The *FFCA* reagent, however, is not specific for iodine but also gives positive reactions with certain reducing substances[6]. The ceric-arsenite reaction has several disadvantages and is generally not used in the spraying technique[9].

The diazotized sulphanilic acid reagent can be used for the simultaneous identification of iodide as well as for iodinated amino-acids by spraying first with the diazotized sulphanilic acid, in which case a greyish or reddish-brown (depending on the concentration of iodide ions) spot locates iodide. This spot begins to disappear conveniently after 30 sec. and completely vanishes after about 2 min., thus leaving sufficient time for it to be marked. While the paper is still moist, 5 per cent (w/v) sodium carbonate is applied and the pinkish spots of the amino-acids appear. The iodine spots develop because of acid oxidation of iodide to iodine in the presence of sodium nitrite, since a mixture of 1·0 *N* hydrochloric acid and sodium nitrite, like sulphanilic acid and sodium nitrite (w/v), also give positive reactions with iodide. If the chromatogram is sprayed with sodium carbonate before it is sprayed with diazotized sulphanilic acid, the acid is neutralized and the iodine spots do not develop. In



a *b*

Fig. 1 (*a*) A two-dimensional chromatogram sprayed with diazotized sulphanilic acid to locate iodide. (*b*) The same chromatogram sprayed with 5 per cent sodium carbonate after the diazotized sulphanilic acid had been applied and the iodide spot had disappeared. Solvent systems: butanol/acetic acid/water (120 : 30 : 50) in one dimension; butanol/dioxan/2 *N* ammonium hydroxide (4 : 1 : 5) in the other dimension. Carriers: 20 μgm. each of iodide, mono-iodotyrosine, diiodotyrosine, triiodothyronine, thyroxine

one- and two-dimensional chromatograms, quantities of 10 μgm. of iodide can still be easily detected by this method. Figs. 1*a* and *b* indicate the use of this test for the simultaneous identification of iodide and iodinated amino-acids.

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BIOCHEMISTRY

Purine and Pyrimidine Metabolism in *Rhodospirillum rubrum*

We have investigated the ability of the photosynthetic bacterium, *Rhodospirillum rubrum*, to deaminate aminopurines and aminopyrimidines.

Washed suspensions in 0·001 *M* phosphate buffer deaminate rapidly cytosine, methylcytosine and adenine to uracil, thymine and hypoxanthine, respectively. The products of the reaction were identified against authentic samples by paper chromatography with a variety of solvents, and the kinetics of the reaction followed either by eluting the spots and measuring the *E* values of the eluates at appropriate wave-lengths or, in the case of the adenine → hypoxanthine and cytosine → uracil conversions, by methods based on differential spectrophotometry. The method of Kalckar[1] was used for the first determination, and for the second determination we devised a similar method by which the amounts of cytosine and uracil could be determined by measuring ΔE at either 280 mμ or 250 mμ at *p*H 1·0. The production of 1 μmole of uracil per ml. of solution is indicated by a $\Delta E280$ of − 8·2 and a $\Delta E250$ of + 3·74. The enzyme is soluble (not sedimented at 100,000*g* in cell-free extracts produced by sonic disintegration) with a sharp *p*H optimum for both pyrimidine and purine substrates at 7·0; it deaminates cytosine some ten times faster than adenine and about twice as fast as 5-methylcytosine. The chromatophores show no significant activity.

The specificity of the enzyme is indicated in Table 1. This shows that while guanine and *iso*guanine are probably deaminated to a very limited extent, neither 2,6-diaminopurine nor aza-adenine is attacked. Furthermore, aza-adenine does not inhibit deamination of adenine. With regard to pyrimidines, the specificity of the enzyme is more clear-cut: only those compounds carrying an amino group on position 6 are deaminated.

Table 1. Specificity of the Purine/Pyrimidine Deaminase of *Rhodospirillum rubrum*

Purines		Pyrimidines	
Compound	Activity	Compound	Activity
Adenine	+	Cytosine	+
Guanine	?	5-Methylcytosine	+
*iso*Guanine	?	*iso*Cytosine	−
Aza-adenine	−	2-Aminopyrimidine	−
2,6-Diaminopurine	−	2,5-Diamino-4,6-dihydroxypyrimidine	−

Adenosine, when incubated with washed cells of *R. rubrum*, disappears rapidly; no free inosine is ever detected, but after long incubation (over 4 hr.) traces of hypoxanthine are noted; this suggests that adenosine is incorporated rapidly *in toto* into cellular material. Deoxyadenosine, on the other hand, is converted mainly into deoxyinosine; small traces of hypoxanthine are observed after prolonged incubation. Cytidine was not attacked in any way; deoxycytidine was not examined.

An attempt was made to demonstrate the presence of a deoxynucleoside transferase in *R. rubrum*. This enzyme will catalyse reactions similar to that recorded in (1). If such a reaction occurred, the adenine produced would be rapidly deaminated by the enzyme just discussed, while if it did not occur, the results of the reaction would be no different from the results observed with deoxyadenosine alone, that is, deoxyinosine would be the main product. Under our conditions, no deoxynucleoside transferase activity could be demonstrated in system (1), or when uracil was replaced by thymine and xanthine.

deoxyadenosine + uracil ⇌ adenine + uracil deoxyriboside (1)

Hug and Werkman[2] found that whole cells and cell-free preparations of *R. rubrum* grown in darkness or light contain an active transaminase. In preliminary experiments in which adenine or cytosine were incubated with α-ketoglutarate we found that α-ketoglutarate had been aminated and adenine (or cytosine) had been deaminated. However, it soon

emerged that this was not a new transaminase reaction, but that α-ketoglutarate was being aminated by the ammonium ions liberated during the deamination of adenine. Incubation of a washed suspension of *R. rubrum* with α-ketoglutarate and ammonium sulphate resulted in a marked formation of glutamate; under our conditions this was greater than when L-alanine was the amino donor.

It is hoped to publish this work in detail shortly. We thank the Royal Society for a grant to purchase a sonic disintegrator and Sigma Chemical Co. for a gift of purines and pyrimidines. This work was carried out in the Biochemistry Department, University of Liverpool. One of us (P. N. A. P.) carried out this work during the tenure of a Royal Thai Air Force scholarship.

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Intracellular Glycosidases of Dextran-producing Bacteria

Extensive work on the mechanism of dextran production from sucrose by bacterial extracellular dextransucrase has not, so far, clearly established the identity of the initial acceptors involved *in vivo* in dextran chain initiation[1,2]. As a step to obtaining such information we have examined the intracellular glycosidase and transferase activities of two dextran-producing bacteria. These were *Leuconostoc mesenteroides* (NRRL *B*512, NCIB 8590; from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen) and *Streptococcus bovis* (strain I)[3].

Each organism was grown in an appropriate medium[3,4] containing sucrose (10 per cent w/v) plus sodium acetate (0·147 *M*). *Leuconostoc* cultures were incubated for 24 hr. at 25° and *Streptococcus* cultures for 5–6 hr. at 38°. During this time good growth occurred (*p*H fall from 7·0 to 5·8–6·0) but the dextran content remained sufficiently low to permit the cells to be collected easily. The centrifuged cells were washed twice in acetate buffer (0·05 *M*, *p*H 5·2) and disintegrated in 5–10 ml. of the same buffer at 2° in a Nossal cell-smasher[5]. After removal of cell debris by centrifugation the extracts were diluted with more buffer to give, from the cells from each litre of culture, 25 ml. of extract which was kept at 2° under toluene.

Digests were prepared containing cell-extract (0·25 ml.), buffer (0·25 ml., *Leuconostoc* extracts, acetate, 0·05 *M*, *p*H 5·2; *Streptococcus* extracts, phosphate–citrate, 0·15 *M*, *p*H 6·5) and sugar (10 mgm.). All sugars used were shown, by paper chromatography, to be pure or to contain only traces of sugar impurities. Controls omitting added sugar and cell-extract respectively were also prepared. The digests and controls were incubated for 24 hr. at 25° or 38° C. and analysed by paper chromatography, both before and after incubation, using ethyl acetate/water/pyridine (2 : 2 : 1) solvent and silver nitrate spray reagent[6].

The controls showed that neither the cell-extracts nor the added sugars alone produced any new sugars during incubation. Preliminary *iso*maltose, arbutin and sucrose digests, at various *p*H values, showed that the *Leuconostoc* carbohydrases were active over the *p*H-range 5·0–7·0, with maximum activity in the range 5·0–5·5, whereas the *Streptococcus* extracts were only active in the range 6·0–7·0, with maximum activity at about 6·5. The degree of hydrolysis of various sugars by the cell-extracts is shown in Table 1. In the disaccharide digests where strong hydrolysis occurred, there was also oligosaccharide formation by transferase action. *L. mesenteroides* was also grown in a 2 per cent maltose medium after passage through two maltose sub-cultures. The extract from these cells hydrolysed the same sugars as the sucrose cell-extracts at similar rates except for maltose, which was more rapidly attacked (spot-intensity of hydrolysis product + + +).

Table 1. Hydrolysis of Sugars by Extracts from Dextran-producing Cells of *Leuconostoc mesenteroides* and *Streptococcus bovis*

Sugar	Degree of hydrolysis*		
	L. mesenteroides extracts at *p*H 5·2		*S. bovis* extracts at *p*H 6·5
	25°	38°	38°
*iso*Maltose	+ + + + +	+ +	+ + + + +
*iso*Maltotriose	+ +	–	+ + + +
*iso*Maltulose	+		–
Melibiose	+ + +	–	+ + + +
Panose	+ +		+ + +
Maltose, leucrose	+	–	–
Nigerose, turanose, αα′-trehalose, methyl-α-glucoside	–	–	–
Sophorose	+ + + +		–
Gentiobiose, lactose, cellobiose	–	–	–
Sucrose	+ + + + + +	+ + + + + +	+ + + + + +
Raffinose	–	–	+ + + + + +
Melezitose	–	–	–
Arbutin, salicin, æsculin, linamarin, lotaustralin	+ + + + + +	+ + + + + +	–
Amygdalin, hesperidin, rutin	–	–	–

* Degree of hydrolysis assessed visually from the spot intensity, on the chromatograms, of hydrolysis products on the scale: + + + + + +, intense spot with substrate completely hydrolysed; down to +, barely detectable spot, trace hydrolysis only and –, no hydrolysis. Blank space, not tested.

Strong invertase activity was present in all cell-extracts, although it is evident that the *Leuconostoc* invertase is much more specific with respect to substrate than the *Streptococcus* one. Chromatographic analyses of hydrolysates of small amounts of the isolated sugars suggest that the oligosaccharides produced in the cell extract-sucrose digests are the well-known fructosylsucrose trisaccharides normally produced by invertase action.

The only α-glucosidase present to any extent in the cell extracts of both bacteria was an apparently constitutive α-1,6-glucosidase. The presence of this enzyme, which hydrolyses the repeating unit of dextran (*iso*maltose), in two unrelated dextran-producing bacteria is striking. The β-glucosidase activity in the *Leuconostoc* extracts also seems unusual in that it was only able to hydrolyse the β-link in glucosides with monosaccharide and aglycone residues and did not attack disaccharide derivatives of this type. Further, apart from sophorose, it was without action on β-linked reducing disaccharides. Attempts are being made to separate purer fractions of the β-glucosidase and α-1,6-glucosidase from the cell extracts.

XUM

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Isolation of Nigerose and Kojibiose from Dextrans

The occurrence of 1,3-α linkage in some dextrans has already been suggested by many workers[1–5]. Barker *et al.*[3,4] have fractionated the partial acid hydrolysate of the dextran produced by *Betacoccus arabinosaseous* (Birmingham strain) on a charcoal column and recognized the presence of a trace of nigerose by paper chromatography. Jeanes *et al.*[5] have characterized dextrans from 96 strains of bacteria by periodate oxidation analysis and classified them into several groups on the basis of the proportions of 1,3 like linkages. According to their results, 29 of them appeared to contain more than 10 per cent 1,3-linkages and a few of them showed an extremely high content of 1,3-linkages (more than 30 per cent).

In 1956, Scott *et al.*[6] pointed out the presence of 1,2-linkage in some dextrans from the optical rotational shifts due to formation of a cuprammonium complex. According to their report, a few of them (for example, that from *Leuconostoc mesenteroides* NRRL *B*–1299 or *B*–1399) contain more than 30 per cent 1,2-linkages.

In spite of these observations, however, no oligo- or di-saccharides containing the heterogeneous glucosidic linkages have so far been isolated from the hydrolysates of dextrans. This may be due to the difference of the stability of 1,6- and other linkages against acid hydrolysis; the 1,6-linkage is much more stable than the other linkages. Therefore, by the usual method of hydrolysis in aqueous acid solution, heterogeneous linkages are more rapidly hydrolysed than the 1,6-linkage.

We have now found that acetolysis is more effective for the isolation of the heterogeneous linkages from dextrans. In contrast with hydrolysis, the 1,6-linkage is less stable to acetolysis, whereas the other linkages such as 1,2- and 1,3- are more resistant. For example, when the acetates of 1,6-linked isomaltose and 1,3-linked nigerose were subjected to acetolysis using the method to be described, the former was entirely cleaved into glucose in 6 hr. at 25° C., whereas the latter remained unchanged. By this method, we have now been able to isolate nigerose and kojibiose from the acetolysates of some dextrans. The dextrans used were prepared by the usual method from *Leuconostoc mesenteroides* NRRL *B*–421 and *B*–1299. The acetolysis reagent was a mixture of acetic anhydride, glacial acetic acid and concentrated sulphuric acid (24:16:3 by vol.). 20 gm. of dried and powdered dextran was suspended in 172 ml. of the above acetolysis reagent and kept at 30° C. for 7 days. The reaction mixture was then heated on a water-bath at 80° C. for 30 min. and poured into crushed ice. After neutralization with sodium carbonate, the whole solution was extracted with chloroform. On removal of the solvent, syrupy mixed acetates were obtained, which were deacetylated with sodium methoxide as usual. Yields of the acetolysates were 10–15 gm. from 20 gm. of the original dextrans. To ascertain if a reversible reaction had occurred, anhydrous glucose was treated exactly as described above; but no reversion product was detected by paper chromatography. The acetolysate of the dextran from *Leuconostoc mesenteroides* NRRL *B*–421 (29·7 gm.) was fractionated on a charcoal/'Celite' column by the gradient elution method. From the 5 per cent ethanol effluent, 7·8 gm. of nigerose and only 0·4 gm. of isomaltose were obtained. Nigerose was characterized as its crystalline octaacetate, melting point 149° C., undepressed on admixture with an authentic specimen.

In the same manner, 7·5 gm. of kojibiose and 1·0 gm. of nigerose were obtained from 22·0 gm. of the acetolysate of the dextran of *Leuconostoc mesenteroides* NRRL *B*–1299. Kojibiose was also characterized as its α- and β-octaacetates: α-kojibiose octaacetate, melting point 166° C.; β-kojibiose octaacetate, melting point 118° C. Neither acetates showed any depression on admixture with authentic specimens.

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Role of Lipid in the Hardening of the Cuticle in the Silkworm, *Bombyx mori*

Malek[1], and Dennell and Malek[2], have stated that the cuticle of *Periplaneta* becomes impregnated with a sterol and a protein rich in tyrosine, and Dennell[3] has given evidence that, in the development of the puparium of *Calliphora*, destruction of localized areas of the epidermis prevents the passage of sterol to the cuticle and also causes lack of tanning of the cuticle immediately above the operated areas.

Table 1. Hardening and Pigmentation of the Pupal Cuticle treated with Some Organic Solvents at 25° C.

Solvent	Pigmentation	Hardening	Remarks
Methanol	(±)* ~ +	(±) ~ +	10–20 per cent of treated pupæ survived
Ethanol	(±) ~ +	(±) ~ +	,, ,,
Butanol	− ~ (±)	−	Died in a few hours
Acetone	(±) ~ +	(±) ~ +	10–20 per cent of treated pupæ survived
Ether	± ~ +	− ~ ±	Died in 2–3 days
Chloroform	− ~ (±)	−	Died in a few hours
Carbon tetrachloride	− ~ (±)	−	,, ,,
Benzol	− ~ (±)	−	,, ,,
Xylol	− ~ (±)	−	,, ,,
Ethyl acetate	− ~ (±)	−	,, ,,

* Brackets indicate that the effect was not usual. + = pigmentation (or hardening); ± = slight pigmentation (or hardening); − = no pigmentation (or hardening).

The lipid layer of the epicuticle of very young and soft pupæ of the silkworm *Bombyx mori* (race *N*124 × *C*124) has been removed within 30–150 min. of pupation either by immersion in ether for 10–15 sec. or by rubbing with cotton wetted with ether. After these treatments, pigmentation of the treated parts proceeds only slowly or imperfectly, and the cuticle remains unhardened. The pupæ thus treated shrank due to the evaporation of water, but some remained alive for a few days and showed an almost normal amber colour in the whole body or only in the region of the dorsal blood vessel (Fig. 1, *b* and *c*). This did not happen when methyl alcohol, ethyl alcohol, or acetone was substituted for ether. Pupæ treated with chloroform, xylol, benzene, ethyl acetate or carbon tetrachloride died within a few hours (Table 1).

Histochemical observation shows that the cuticle treated with ether is almost deprived of epicuticle lipid (see ref. 4). Replacement of the lipid by the ether extract from the cuticle or by 'Vaseline' prevented the pupæ from shrinking, but did not cause cuticle hardening to proceed.

In agreement with Malek[1], and Jones and Sinclair[5], these results indicate that hardening and pigmentation of the cuticle are independent processes. However, it is not clear whether the lipid is directly involved in the hardening process, or whether it merely plays some part in facilitating the process. Microanalysis by paper chromatography has shown that the ether extracts from cuticles contain no phenolic substances.

I thank Prof. K. Hasegawa for his advice.

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(*a*) (*b*) (*c*)

Fig. 1. Pupæ 6 hr. after ether treatment. (*a*) Control; (*b*) and (*c*) treated pupæ

Action of Neuraminidase on Haptoglobin

In the course of work on the chemical structure of haptoglobin (Hp)[1], we have had occasion to investigate the action of neuraminidase on the Hp and on the complex of Hp with hæmoglobin (Hb-Hp). The neuraminidase employed was prepared as described by Mayron and Rafelson[2,3] from influenza virus, Asian strain (Jap. 305), and is free of proteolytic activity and glucosidase activities other than neuraminidase action. The Hp II preparations utilized were prepared as previously described[4], and are considered to approach the stage of theoretical purity. Such preparations contain 5·1 ± 0·1 per cent N-acetylneuraminic acid (NANA)[1]. The liberation of NANA from Hp II and Hb-Hp II was determined by the method of Warren[5] using three-times crystallized NANA as a standard. A typical enzyme incubation at 37° employed 2–5 mgm. of Hp II or Hb-Hp II and 0·1 to 0·3 unit of neuraminidase in 0·1 *M* phosphate buffer, *p*H 6·5, total volume 2–3 ml. One unit of enzyme activity is defined as that amount of enzyme which liberates 1 mgm. of NANA from an excess of neuramin-lactose in 1 hr. at 37°, *p*H 6·5.

Fig. 1 shows the percentage of NANA liberated as a function of time from Hp II and Hb-Hp II by the

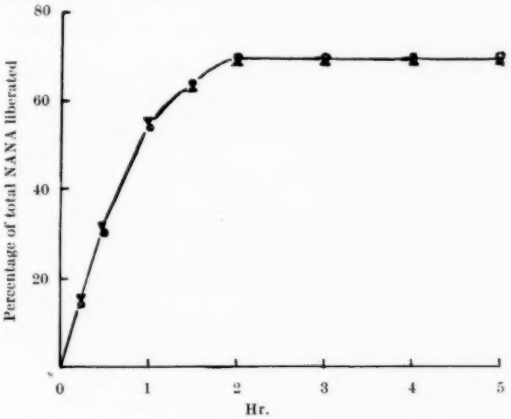

Fig. 1. Action of neuraminidase on Hp II and Hb-Hp. ○, Hp II; ▲, Hb-Hp II

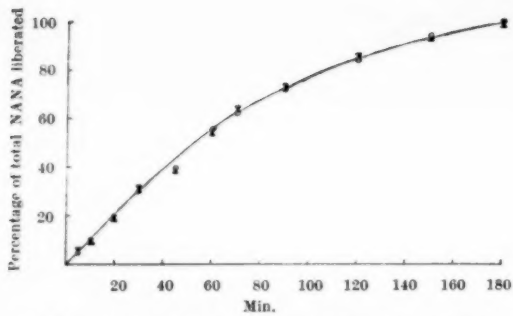

Fig. 2. Liberation of NANA from Hp 70 per cent NANA and from Hb–Hp 70 per cent NANA by 0·01 *N* hydrochloric acid at 80°. ○, Hp II 70 per cent NANA; ▲, Hb-Hp 70 per cent NANA

viral neuraminidase. It may be seen that the time-course of appearance of free NANA from the two substrates was identical, and that a maximum of 70 per cent of the total NANA was liberated in both cases. The *p*H optimum (*p*H 4·2) and the total peroxidase activity of the untreated complex (Hb-Hp), of the complex formed from untreated Hp II and of the complex formed from treated Hp (Hb-Hp 70 per cent NANA) were all identical. The conclusion which may be drawn from these results is that the 70 per cent of the NANA removed from Hp II and from Hb-Hp II participates neither in the formation of the Hb-Hp complex nor in the peroxidase activity of the complex. The possibility that the 30 per cent of NANA which is resistant to the action of neuraminidase may play a part in both the formation of the complex and in its peroxidase action cannot be excluded, although the following experiment suggests that this NANA is not involved in the formation of the Hb-Hp II complex.

Fig. 2 gives the results of an experiment in which the time-course of liberation of NANA by 0·01 *N* hydrochloric acid at 80° from Hp II previously treated with neuraminidase was compared with the liberation of NANA from the complex formed from this pretreated Hp II. The kinetic curves for the liberation of NANA from both substances were identical. These results are interpreted to suggest that the portion of NANA not liberated by the action of neuraminidase is not involved in the formation of the Hb-Hp II complex. If the 30 per cent of the NANA which is resistant to neuraminidase action were involved in the formation of the complex, it might be expected that kinetic differences in the release of NANA should have been observed.

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Low-Angle Light Scattering and the Molecular Weight of Deoxyribonucleic Acid

It has been known for some time that the molecular weights obtained for deoxyribonucleic acid (DNA) by present-day techniques in light-scattering may be in error. This situation is not the result of theoretical barriers, but rather, the very considerable experimental problems to be solved in making reliable measurements of scattering below angles of 25°. Accordingly, considerable effort has been made to assess by indirect methods the validity of light-scattering molecular weights determined by extrapolation of data which extend to no lower than 25°. Doty, Geiduschek and Holtzer and more recently Butler *et al.* have given[1-3] detailed critical discussions of the nature of the indirect evidence and the remaining ambiguities.

In the case of molecules as large as DNA, measurements of light scattering must be made down to the angular region[1] of 6–10° to enable a reliable extrapolation to zero angle. Such measurements are necessary to determine the molecular weight of DNA and other very large macromolecules without appeal to any molecular models, but the experimental difficulties are so great that Doty has commented that ". . . there is no likelihood of reaching this range [6–10°] because of practical difficulties".

The difficulties are, indeed, very great, but not insurmountable. I wish to report here measurements made on the scattering of a sample of calf-thymus DNA (prepared by a modification of the method of Signer and Schwander[4]) which sharply increase the likelihood of achieving reliable results at low angles. The measurements extend to 9°.

The scattering was measured in an instrument of my design the calibration and precision of which have been described[5] earlier. Its calibration constant determined by measurements on 'Ludox' is considered to be accurate to within a probable error of ± 0·3 per cent.

The results of the measurements are illustrated in Fig. 1. The plot has been made according to the equation:

$$\frac{Kc}{R_{\theta,u}} = \frac{1}{M_w}\left(1 + \frac{16\pi^2 n_0^2}{3\lambda_0^2}[\rho_G]^2 \sin^2(\theta/2) + \ldots\right) + 2A_2Q(\theta)c$$

where $R_{\theta,u}$ is the reduced intensity. c is the concentration (gm./ml.), K is $[2\pi^2 n^2(\mathrm{d}n/\mathrm{d}c)]^2/N_0\lambda_0^4$, M_w is the weight-average molecular weight, $[\rho_G]$ is the average radius of gyration, n is the refractive index of the solution and λ_0 is wave-length of the incident light *in vacuo* (here 4358 Å.).

The results can be fitted over the entire angular range of 9–45° by a straight line with a probable error of ± 4 per cent. Least-square calculations give $(Kc/R_{\theta,u})_{\theta=0} = 8{\cdot}2 \times 10^{-3}$ if all the data (9–45°) are included. The result is $7{\cdot}9 \times 10^{-3}$ when only the angular range 25–45° is considered and $8{\cdot}4 \times 10^{-3}$ for the range 9–17°.

The weight-average molecular weight obtained from the range 9–45° is $12{\cdot}2 \times 10^6$ and is independent of any assumptions concerning the shape. The

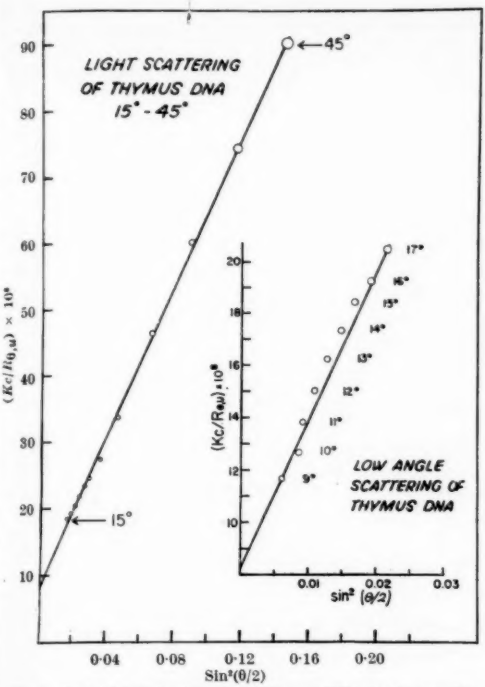

Fig. 1. The light-scattering of calf-thymus DNA in the angular region 9–45°. The straight line in both the main figure and the inset is that calculated by least squares for the entire angular region shown. The concentration of DNA was $3{\cdot}12 \times 10^{-5}$ gm./ml.

average radius of gyration, determined in the usual fashion, is 3700 Å. The reduced viscosity at zero shear for the sample reported on here was 45 as determined by measurements in a three-bulb viscometer.

The light-scattering data at higher angles, 50–135°, display a downward curvature which can be fitted reasonably well by a straight line in the range 60–135°. Extrapolated to zero angle, this line yields a value of 40×10^{-5} for the intercept. The interpretation[1–3] of the curvature is complicated: it may arise from polydispersity of molecular weights; it may be the result of variability in the shape of the molecules, nor is a combination of these two factors excluded. At present there is no unambiguous method of determining these properties independently for DNA in solution.

The experimental work reported here was completed at the Virus Laboratory of the University of California, Berkeley. It was supported partly by contract *NR* 121–175 between the Office of Naval Research and the University of California. A more detailed report of the experiments will be available shortly.

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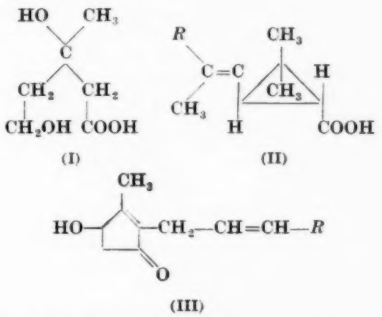
Biosynthesis of the Pyrethrins

RECENT work[1] has indicated that mevalonic acid (I) is an intermediate in the biosynthesis of terpenoid compounds. We now wish to report some experiments which indicate that this acid is also involved in the biosynthesis of the 'pyrethrins', the insecticidal constituents of the pyrethrum plant, *Chrysanthemum cinerariaefolium*. The 'pyrethrins' are a mixture of four closely related esters[2] derived from two acids, chrysanthemum monocarboxylic acid (II, R = —Me) and pyrethric acid (II, R = —COOMe), and two keto-alcohols, pyrethrolone (III, R = —CH=CH_2) and cinerolone (III, R = —CH_3). It is customary to refer to the mixture of the two esters derived from chrysanthemum monocarboxylic acid and the keto-alcohols as 'pyrethrin I' and to that from pyrethric acid as 'pyrethrin II'. The two acids have carbon skeletons which can be built up from two isoprene units, and thus might be expected to be derived from mevalonic acid. The two keto-alcohols cannot be so regarded, but it is of interest to note that the methylpentenone ring could be formed in theory by the cyclization of mevalonic acid. We have now shown that this theoretically attractive route is not employed by the plant.



Because the pyrethrins are concentrated in the achenes[2] experiments on the incorporation of labelled compounds have been done on the flower head. Preliminary work with aqueous solutions of 2-^{14}C-acetic acid and DL-2-^{14}C-mevalonic acid showed that these two compounds moved slowly up the flower stem to the intact flower. A small amount of radioactivity was incorporated into the pyrethrins by this technique. A better incorporation was obtained by dropping an aqueous solution of the acid (I) containing a little detergent on the disk florets of the open flower. Finally, the highest incorporation was obtained by vacuum infiltration[4] of the substrate on ovules dissected from half-open flowers. This observation suggests that the pyrethrins are synthesized as well as stored in the achenes. It has also been found that the ovules from unopened and therefore unfertilized disk florets are slightly better at incorporating mevalonic acid into the pyrethrins than ovules from opened disk florets. This would seem to suggest, contrary to previous indications[5], that fertilization does not increase pyrethrin synthesis in the flower head.

The total pyrethrins were extracted from the plant tissue with acetone, and 'pyrethrins I and II' isolated by chromatography on silica gel/plaster of Paris[6]. The 2,4-dinitrophenylhydrazones of these compounds were prepared[7,8] and recrystallized to constant specific radioactivity. These derivatives were

counted as thin films using an end-window counter. In a typical experiment DL-2-^{14}C-mevalonic acid (2 mgm., 2 μc.) was added to isolated ovules (2 gm.); after 24 hr. the incorporation of radioactivity into the pyrethrins had reached a maximum, corresponding to utilization of 1·4 per cent of the natural (+) isomer[9] of mevalonic acid. In a comparative experiment 2-^{14}C-acetic acid was incorporated to a much smaller extent, approximately one-tenth that of mevalonic acid.

Under strongly acid conditions in methanol 'pyrethrin II' yields with 2,4-dinitrophenylhydrazine the derivative of pyrethrolone methyl ether[7]. Using this method of splitting the esters in 'pyrethrin II', it has been found that while acetic acid is incorporated into the cyclopentenone ring, mevalonic acid is not. Thus, by difference, it is concluded that mevalonic acid is only incorporated into the terpene-like acids (II) of the pyrethrins. The exact location of the C-2 atom of mevalonic acid has not yet been determined; but it would seem very likely that it would be in the geminal methyl groups of the cyclopropane ring in both acids, and in a similar position in the isopropylidene group of 'pyrethrin I'. On the other hand, since the biosynthesis of the pentenones (III) does not involve mevalonic acid, it would seem to be either an example of the acetate route of biosynthesis that has been suggested by Birch and co-workers[10], or else to involve a precursor of mevalonic acid such as mevaldic acid[11] or 3-hydroxy-3-methyl-glutaric acid[12].

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Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by Plants

It has been inferred from feeding-experiments that cress seedlings[1], and also pea and mung bean mitochondria[2], are capable of converting L-galactono-γ-lactone into L-ascorbic acid. Similar results were obtained with mitochondria from cabbage leaves and cauliflower florets, and a relatively specific L-galactono-γ-lactone dehydrogenase was isolated from the latter[3]. The results (Table 1) confirm that this system may not be uncommon in the higher plants; *Sphagnum*, *Spirogyra* and *Psalliota*‡ gave inconclusive results. The figures in Table 1 are either the mean of duplicate estimations, or are taken from single, extended experiments which show how the concentration of ascorbic acid in the tissue varies during the period of feeding. Fig. 1 shows the results of one such extended experiment with parsley, in which the concentration of ascorbic acid in the laminæ fed with the lactone rose, during a period of 190 hr., from 1,582 to 12,010 mgm./100 gm. dry weight, that is, to approximately 12 per cent of the dry weight. During the same period, the value for the controls in de-ionized water fell gradually to 1,120 mgm./100 gm. dry weight, and at this time the experimental laminæ possessed more than ten times the concentration of ascorbic acid in the controls.

Table 1. Effect of Feeding with L-Galactono-γ-Lactone on the Concentration of Ascorbic Acid in Certain Plant Parts

Plant material	Period of feeding (hr.)	Concentration of ascorbic acid (mgm./100 gm. dry wt.)		
		Initial value	Control samples in water	Experimental samples in lactone
*Slices of flesh from full-size, green hips of				
Rosa sherardi	66	3310	3540	5110
R. canina	66	1421	1500	2710
R. arvensis	66	342	344	1326
†Entire leaves of				
Parsley (*Petroselinum crispum*)	48	920	850	2450
Scurvy-grass (*Cochlearia officinalis*)	48	900	560	2650
Watercress (*Nasturtium officinale*)	24	620	650	2700
‡Procumbent yellow sorrel (*Oxalis corniculata*)	24	751	736	1587
‡Kentucky blue-grass (*Poa pratensis*)	33	331	304	1936
‡Maidenhair fern (*Adiantum capillus-veneris*)	56	531	397	1192

* The tissue slices were suspended and aerated at 25° C. either in de-ionized water or in a 0·5 per cent (w/v) solution of L-galactono-γ-lactone.
† The petioles of the leaves (except for watercress) were stood either in de-ionized water or in the solution of the lactone. The watercress leaves were floated on the appropriate liquid in Petri dishes.
‡ North American material, kept during feeding in a constant-temperature room at a temperature of approximately 22° C. under a daily regime of 14 hr. light and 10 hr. darkness; light intensity approximately 500 ft.-candles. The remaining material was British and was kept during feeding on a window-bench in the normal laboratory, with a diurnal cycle of approximately 11 hr. light and 13 hr. darkness.

Three pathways have been proposed for the synthesis of ascorbic acid in plants: (i) from D-glucose via D-glucurono-γ-lactone[1]; (ii) from

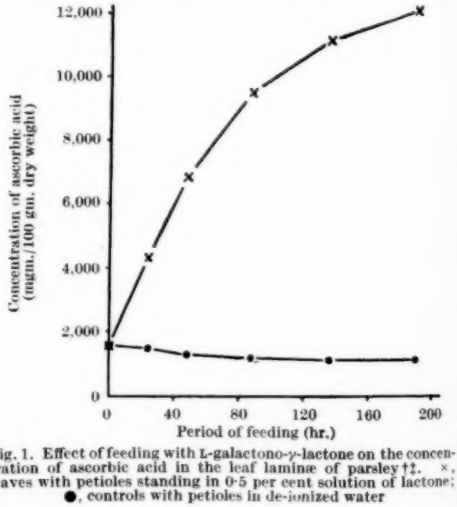

Fig. 1. Effect of feeding with L-galactono-γ-lactone on the concentration of ascorbic acid in the leaf laminæ of parsley †‡. ×, leaves with petioles standing in 0·5 per cent solution of lactone; ●, controls with petioles in de-ionized water

D-galactose via L-galactono-γ-lactone[1], and (iii) from D-glucose via glucose-6-phosphate and other phosphorylated intermediates[4]. Feeding with carbon-14-labelled compounds has led to the conclusion that, although D-glucurono-γ-lactone fed to strawberries may serve as a precursor in the synthesis of ascorbic acid[5], only pathway (iii) is involved in normal synthesis in this fruit and in cress seedlings[4,6]. On the basis of this view, any functional significance of the active system capable of converting L-galactono-γ-lactone into ascorbic acid remains to be elucidated; this problem is now being studied.

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A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes

In previous papers, Nakao *et al.*[1,2] reported that adenosine triphosphate (ATP) is regenerated in erythrocytes stored for more than several weeks or treated with fluoride[3], when the cells are incubated with nucleoside and adenine at 37° C. In these aged or fluoride-treated cells, most of the nucleotides and other acid-soluble phosphate esters were decomposed almost completely and energy metabolism almost entirely stopped. To elucidate the mechanism of adenosine triphosphate synthesis in such erythrocytes, the following observations were carried out:

(1) As reported previously, inorganic phosphorus-32 ($i^{32}P$) and adenine-8-^{14}C were incorporated into ATP in long-stored erythrocytes after incubation with inosine, adenine-^{14}C and inorganic phosphorus-32. The incorporation of inorganic phosphorus-32 into ATP is inhibited by sodium-fluoride, mono-iodoacetate and arsenate, and adenine can be replaced by no other purine or pyrimidine bases, while inosine can be replaced by adenosine, deoxyadenosine, guanosine and deoxyguanosine though pyrimidine nucleosides were not effective[2]. These results show that ATP was synthesized from added adenine and added inorganic phosphate, and that glycolysis system and nucleoside phosphorylase seem to have some connexion with synthesis of ATP.

(2) The time-course of ATP generation during incubation with adenine and inosine in erythrocytes previously stored for 15 wk. or treated with sodium fluoride for several hours is shown in Fig. 1. The erythrocytes stored for 15 wk. or longer and those treated with sodium fluoride for 8 hr. showed a time-lag, in contrast to those stored for shorter periods or not treated with sodium fluoride.

(3) Hæmolysate obtained from fresh human erythrocytes was previously dialysed for 24 hr. at 4° C. and then passed through a 'Dowex 1' column in order to eliminate thoroughly all of the acid-soluble phosphorus compounds, using inorganic phosphorus-32 as an indicator. The incubation was carried out with a reaction mixture containing inorganic phosphate, magnesium, nicotinamide, adenine-8-^{14}C, either inosine or ribose-5-phosphate (R5P), and the pre-treated hæmolysate. When a trace amount of ATP was added to the medium, carbon-14 was found in adenosine monophosphate (AMP), but not in ATP, while no radioactivity was found either in AMP or in ATP, when ATP was not added to the reaction mixture. The time-course of the incorporation of adenine into nucleotide is illustrated in Fig. 2. It shows a remarkable increase in adenine-^{14}C incorporation with a time-lag in the case where diphosphopyridine nucleotide (DPN) was added and this time-lag disappeared when a greater amount of ATP was added to the medium. The results obtained from experiments (2) and (3) indicate that the existence of the lag phase was neither due to the inhibitory effect of a contaminating substance nor due to the induced

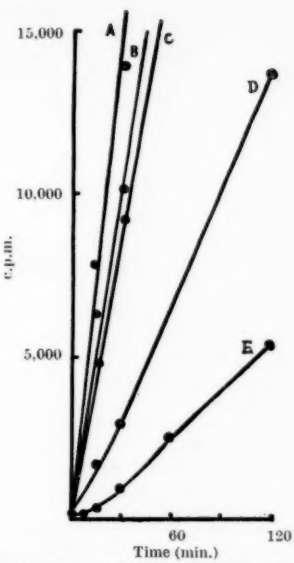

Fig. 1. Incorporation of adenine-8-^{14}C into nucleotide by intact erythrocytes. Reaction mixture contained 0·1 μmole (46·400 c.p.m.) of adenine-^{14}C, 5 μmoles of inosine, 0·7 ml. of intact cells, 2 μmoles of magnesium chloride, 10 μmoles of inorganic phosphate and 30 μmoles of *tris* buffer (*p*H 7·5); total volume, 1·32 ml.; temperature, 37° C. After incubation, the barium salt of nucleotides was isolated according to Kornberg (ref. 7) and its radioactivity was measured by means of a gas-flow counter. Pre-incubation with sodium fluoride of the blood stored for 4 wk. at 4° C. was carried out for 0(*A*), 2(*B*), 4(*C*), 8(*D*) hr. at 37° C. and (*E*) shows blood stored for 15 wk. at 4° C., in which no further pre-incubation was performed

Table 1. Synthesis of ATP by Pre-treated Hæmolysate

Reaction mixture: Inosine, 50 μmoles, adenine, 5 μmoles, magnesium chloride, 10 μmoles, inorganic phosphate, 50 μmoles, DPN, 0·05 μmole, ATP, 0·05 μmole, nicotinamide, 66 μmoles, hæmolysate pre-treated as shown in the footnote of Fig. 2, 2·5 ml., total volume, 6·60 ml. Incubation was carried out for 60 min. at 37° C. After incubation, the acid-soluble fraction was analysed by Cohn and Carter's column chromatography on 'Dowex 1(Cl)'

	Initial (μmole)	Final (μmole)	Difference (μmole)
Inosine	50·0	30·0	−20·0
Adenine	5·0	4·18	− 0·82
ATP	0·05	0·43	+ 0·38
ADP	–	0·51	+ 0·51
AMP	–	0·087	+ 0·087
Lactic acid	0	10·8	+10·8

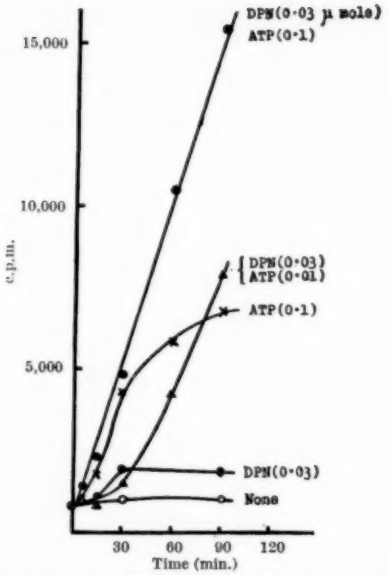

Fig. 2. Incorporation of adenine-8-^{14}C into adenine nucleotide by pre-treated hæmolysate. Reaction mixture contained 1 μmole (94·500 c.p.m.) adenine-8-^{14}C, 10 μmoles of inosine, 2 μmoles of magnesium chloride, 10 μmoles of inorganic phosphate, 10 μmoles of nicotinamide and 0·7 ml. of the pre-treated hæmolysate, namely, dialysed for 24 hr. at 4° C. and passed through 'Dowex 1' column (*Cl*) to eliminate thoroughly all the nucleotides, using inorganic phosphorus-32 as an indicator. The total volume was 1·32 ml. Incubation was carried out at 37° C.

formation of enzyme, but possibly due to the increase of coenzymes or of substrates.

(4) Not only incorporation of adenine-^{14}C into ATP, but also a net increase of ATP was determined by Cohn and Carter's ion-exchange chromatography[4] on 'Dowex 1', after the pre-treated hæmolysate was incubated with adenine, inosine, inorganic phosphate, magnesium, a small amount of DPN and of ATP, as shown in Table 1.

Preiss and Handler[5] reported in 1958 that R5P and ATP are converted to phosphoribosyl pyrophosphate (PRPP), and AMP and then PRPP and adenine yield AMP and inorganic pyrophosphate (PP) in acetone powder extract of human red cells. The presence of powerful adenylate kinase activity in human erythrocytes was reported by Tatibana *et al.*[6]. Moreover, there are a few observations indicating the existence of a considerable amount of glycolytic activity even in erythrocytes stored for more than several weeks. For example, when incubated with inosine, 2,3-diphosphoglyceric acid increased in erythrocytes stored for a long period[1,3] and lactate also increased in the erythrocytes when incubated with inosine and adenine or in hæmolysate when incubated with ATP and inosine. According to these results and the observations of others mentioned above, the mechanism of ATP generation might be explained tentatively as follows:

$$R5P + ATP \rightleftarrows AMP + PRPP \quad (1)$$

$$PRPP + \text{adenine} \rightleftarrows AMP + PP \quad (2)$$

$$2AMP + 2ATP \rightleftarrows 4ADP \quad (3)$$

$$4ADP + nR5P \rightarrow 4ATP + X \quad (4)$$

(Warburg-Dickens system and Embden-Meyerhof system)

$$\text{Sum}: (n+1)\,R5P + \text{adenine} + 3ATP \rightarrow 4ATP + PP + X \quad (5)$$

The rapid formation of R5P from inosine and inorganic phosphate in erythrocytes or in hæmolysate is well established. R5P thus formed reacts with a trace amount of ATP still remaining in the cells, forming PRPP and AMP (1). ADP was synthesized from AMP, which was formed in both reactions (1) and (2), from remaining ATP, by the action of adenylate kinase (3). The ADP is converted to ATP in the course of R5P metabolism through Warburg–Dickens and glycolytic systems (4) (Fig. 3).

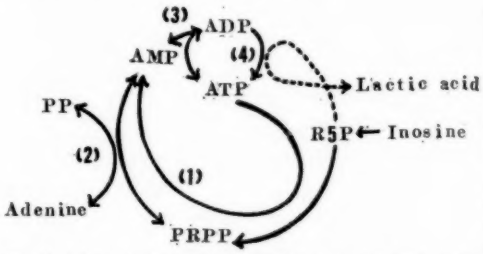

Fig. 3. A hypothesis of ATP regeneration in human erythrocytes

Thus, as a key substance, the trace amount of ATP remaining in the cells makes a cycle of reactions operative in synthesizing a greater amount of ATP: the further the reaction proceeds, the greater the increase in the amount of 'substrate ATP'.

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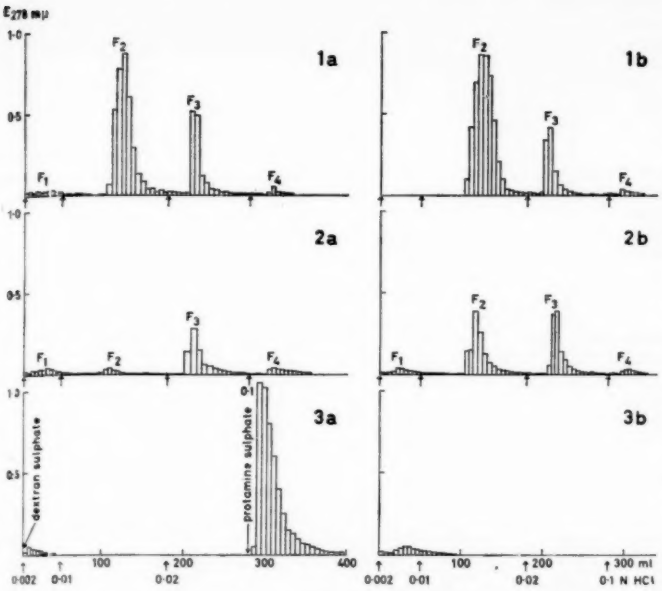
Influence of Polyvalent Ions on Histones isolated from Different Tissues

When dealing with fractionation of histones, we observed that the chromatographic pattern became markedly different depending on the cellular origin of the preparation. We also found that these differences reflected the polysaccharide content of the tissue.

The technique was as follows: deoxynucleoprotein was prepared from fresh frozen tissue through precipitation with 0·15 *N* sodium chloride[1] and an extraction was made with 0·2 *N* hydrochloric acid[1]. The extract, which will contain histones as well as polysaccharides, was gel-filtered at + 4° C. on a 'Sephadex' column, *G* 25, with acetate buffer of *p*H 4·0



Figs. 1–3. Effluent diagrams of histones chromatographed on carboxymethylcellulose columns (15 cm. × 1·4 cm.). All the extracts were applied to the columns in acetate buffer, $I = 0{\cdot}02$ and pH 4·0. Elution with hydrochloric acid of the concentrations marked on the horizontal axis. 1*a*, Run of histones from chicken erythrocytes; 1*b*, the same run as 1*a*, but with protamine sulphate added to the extract before the application to the column; 2*a* and 2*b*, the corresponding runs of histones from calf liver; 3*a*, run of calf thymus histones to which has been added an equivalent amount of dextran sulphate before the extract has been added to the column. Thereafter elution with protamine sulphate. 3*b*, run of protamine sulphate (salmine) alone (from Nutritional Biochemicals Corporation)

and an ion-strength of $0{\cdot}02$ [2,3]. For separation of histone fractions, chromatography on carboxymethylcellulose (CMC) was utilized[4]. The stepwise elution was made with $0{\cdot}002$ *N*, $0{\cdot}01$ *N*, $0{\cdot}02$ *N* and $0{\cdot}1$ *N* hydrochloric acid. The elution was followed through measurements of the extraction at 278 mμ.

Fig. 1*a* shows the chromatographic pattern of extract from chicken erythrocytes during the stepwise elution of the CMC-column. Two distinct and homogeneous fractions of histone were obtained, the peaks F_2 and F_3. Almost the same pattern appeared with extracts from thymus of calf, pig and lamb and from lymph nodules of calf. The ratio F_2/F_3 shows only a slight variation within this material. All these tissues can be regarded as containing a high proportion of histones in relation to polysaccharides. The main difference between the two histone fractions, which are eluted with different strengths of the acid, should be that F_3 is a more basic protein than F_2. Besides, with reference to end-group analyses[4], F_2 and F_3 may represent two qualitatively different types of histones. Fig. 2*a* demonstrates the corresponding elution pattern from calf liver. In this case the histone fraction F_2 is almost non-existent. The same very low ratio F_2/F_3 was also obtained with extracts from other kinds of liver—pig, mouse and chicken—and from whole chicken embryos. All these tissues should have a relatively high amount of polysaccharides in relation to histones. The pattern from extract of calf white blood corpuscles certainly showed both F_2 and F_3, but the ratio F_2/F_3 was low and intermediate between the two groups.

To demonstrate that it was the polysaccharides, which interfered with the histone fractions, an equivalent amount of the acid polysaccharide dextran sulphate was added to thymus-histone attached to the CMC-column. Thereafter no histone could be obtained during the subsequent elution, because the formed histone-polysaccharide complex was not dissociated at the low hydrogen ion concentrations used, Fig. 3*a*. If thereafter an equivalent amount of protamine sulphate was added to the column, the less basic histone is replaced by the more strongly basic protamine in the polysaccharide-complex and the histones can now be eluted quantitatively (Fig. 3*a*). Fig. 3*b* demonstrates the simple chromatographic pattern with protamine alone, which shows that protamine will not interfere with the absorption peaks at 278 mμ of the histone fractions. Figs. 1*b* and 2*b* show the chromatographic pattern after addition of protamine to the histone extract from the two tissues exemplified here. The amount of the F_2-histone has increased, most highly from the polysaccharide-rich extract, 2*b*, while the change in amount of the F_3-histone is only slight. Entirely corresponding results were obtained with the other tissues examined. Thus the F_2-histone has obviously much greater affinity for acid polysaccharides than the F_3-histone.

Thus it seems clear that a quantitative analysis of the native DNA-histone relation cannot be made without a blockading of other acid polyvalent ions, especially of polysaccharide type, and it seems likely that also in the living cell there is the same competition for histones between nucleic acids and acid polysaccharides.

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Side-Reactions during Hydrogenolysis of 2,4-Dinitrophenylhydrazones of Aromatic Keto-Acids

The identification of the keto-acid 2,4-dinitrophenylhydrazones is greatly facilitated by conversion to their respective amino-acids. The most commonly employed procedure is that first noted by Emil Fischer[1] and modified for application to organic systems by Towers *et al.*[2]. This involves low-pressure hydrogenolysis over a platinum catalyst, followed by paper chromatography of the amino-acids produced. The yields of the respective amino-acids vary between 30 and 98 per cent, and in general the conversion is free from side-reactions.

However, the hydrazones of the dicarboxylic acids, α-ketoglutaric and oxalacetic acids, may undergo some decarboxylation during this reaction. Thus, small quantities of alanine are produced from oxalacetic acid, and both α- and γ-aminobutyric acids, in addition to the expected glutamic acid, are found after hydrogenolysis of the 2,4-dinitrophenylhydrazone of α-ketoglutaric acid[2,3].

During recent work on the keto-acid excretion in phenylketonuria and tyrosinosis[4] another potentially important deviation from the theoretical keto→amino-acid conversion could be demonstrated.

Hydrogenolysis of the 2,4-dinitrophenylhydrazone of phenylpyruvic acid employing the conditions recommended by Towers *et al.*[2], namely, a 12-hr. hydrogenation in distilled water at 25° C., using 30 lb./in.² pressure, and 0·2 gm. of platinum oxide catalyst, yielded a mixture of two amino-acids. The main product was phenylalanine, but sizeable amounts of a non-aromatic substance with chromatographic properties of cyclohexylalanine could also be found (Table 1).

Table 1. Chromatographic Behaviour of Hydrogenation Products of Keto-Acid 2,4-Dinitrophenylhydrazones

Keto-acid 2,4-dinitrophenylhydrazone	*Rf* of hydrogenation products 1*	2†	3‡	Relative intensity	Amino-acid formed
Phenylpyruvic acid	0·56	0·37	0·82	3+	Phenylalanine
	0·74	0·53	0·84	1+	Cyclohexylalanine
p-Hydroxyphenylpyruvic acid	0·39	0·28	0·64	Trace	Tyrosine
	0·37	0·32	0·84	1+	Hexahydrotyrosine
	0·73	0·55	0·84	2+	Cyclohexylalanine

Descending chromatography. No. 40 Whatman paper.
* Solvent 1: *n*-butanol (120 parts); glacial acetic acid (30 parts) water (50 parts).
† Solvent 2: 95 per cent ethanol (180 parts); water (10 parts); concentrated ammonium hydroxide (10 parts).
‡ Solvent 3: Liquid phenol (200 parts); concentrated ammonium hydroxide (1 part).
Spots identified by spraying with a 0·2 per cent solution of ninhydrin in acetone containing 2 per cent pyridine.
The chromatographic behaviour of the products was compared with cyclohexylalanine and hexahydrotyrosine prepared according to Waser and Brauchli (ref. 6).

When the 2,4-dinitrophenylhydrazone of *p*-hydroxyphenylpyruvic acid was hydrogenated under similar conditions, approximately equal amounts of hexahydrotyrosine (4-hydroxycyclohexylalanine) and cyclohexylalanine were obtained. Tyrosine, the expected product, was only present in small amounts (Table 1).

The production of the hydrogenated derivatives of phenylalanine and tyrosine represent an unwelcome side-reaction, which, however, is not unexpected.

Many workers have shown that under the conditions employed for hydrogenolysis of the keto-acid 2,4-dinitrophenylhydrazones, hydrogenation of the aromatic ring can also occur. The rate of hydrogenation of the ring has been found to be first order with respect to hydrogen pressure, zero order with respect to the concentration of the compound to be hydrogenated, and directly proportional to the amount of catalyst used[5]. Thus, under the very conditions used in my experiment, hydrogenation of phenylacetic acid proceeds rapidly, 50 per cent of the starting material being converted to cyclohexylacetic acid within 60 min.[5].

Cleavage of the phenolic group has also been described. Waser and Brauchli[6], in subjecting tyrosine to low-pressure hydrogenation over platinum, obtained a mixture of hexahydrotyrosine and cyclohexylalanine.

Factors favouring the replacement of the phenolic group by hydrogen are hydrogenation in an acidic medium[7], relatively low pressures, and a symmetrical structure for the substance to be hydrogenated[8]. Thus, *para*-substitution favours the replacement of OH-groups, while *ortho*-substitution retards it[9].

In concluding, it must be emphasized that side-reactions such as those reported in this communication do not detract from the usefulness of the hydrogenolysis of the α-keto-acid 2,4-dinitrophenylhydrazones as a step towards their identification.

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PHYSIOLOGY

Function of Taste Fibres in the Carp

The carp (*Cyprinus carpio* L.) has a palatal organ supplied by nerve fibres running into the glossopharyngeal nerve. By dissecting and isolating small strands of the nerve branches running to the palate, it has been possible to obtain records of the electric response to the application of different sapid solutions to the mucous surface of the palatal organ.

It was found that such nerve preparations responded to a variety of test solutions such as 0·5 *M* sodium chloride, 0·005 *M* acetic acid, 0·01 *M* quinine and 0·5 *M* sucrose. These nerve preparations also responded very strongly to human saliva, as well as to the mucin covering the scales of the fish. The integrated response of such a nerve-strand to human saliva was generally quite as big as the response to 0·5 *M* sucrose and considerably greater than the response to 0·5 *M* sodium chloride.

A study of single fibre responses revealed that the saliva always stimulated fibres which responded positively to sugar. Fibres which responded to sodium chloride and acid or to quinine, but not to sugar, did not respond to human saliva, but for two exceptional cases. According to their response pattern the palatal chemoceptive fibres seem to be divided into six groups as will be seen from Table 1. Among these different fibres the types I, II and III are most common. So far we have only encountered two fibres of type VI.

Besides chemoceptive fibres and motor fibres, the palatal nerves contain fibres which respond very selectively to mechanical stimulation. The number of such fibres seems, however, to be relatively small

Table 1

Fibre type	Sodium chloride	Acetic acid	Quinine	Sucrose	Saliva
I	+	+	−	+	+
II	+	+	+	+	+
III	−	+	−	+	+
IV	+	(+)	−	−	−
V	−	+	+	−	−
VI	−	+	−	−	+

compared with the chemoceptive fibres. A comparison of the chemoceptive response of the palatal nerves with that from the nerves innervating the lips, the barbels and the gill rakers gives us the impression that the palatal nerves are the most important taste nerves of this species.

That human saliva is such a powerful stimulant of the palatal organ gives us, of course, a certain aspect on the angler's habit of spitting on the bait.

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Pharmacology of the Alkaloidal Fraction from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth.

A PHARMACOLOGICAL screening programme of extracts of plants used as folk medicines in Jamaica for the preparation of the so-called 'bush teas' is being carried out in collaboration with a group under Prof. L. J. Haynes at the Department of Chemistry, and with Dr. P. C. Feng at the Department of Physiology, University College of the West Indies, Jamaica. The shade tree, *Pithecolobium samath* Benth., was selected for detailed study. The main alkaloid from its bark, pithecolobine, had already been isolated by Wiesner *et al.*[1], who have given its partial structure[2].

A crude alkaloidal fraction was isolated from the powdered bark of the shade tree by extraction with dilute hydrochloric acid. The extract was made alkaline and then washed with chloroform, and these washings were then extracted with dilute hydrochloric acid. The acid extract was made alkaline and re-extracted with chloroform. The chloroform was evaporated to give an oil. Paper chromatography of this oil in several solvent systems showed one major component, presumably identical to pithecolobine, and only traces of a second component were disclosed with Dragendorf's reagent[3].

This fraction (referred to as pithecolobine) when injected intraperitoneally into mice (40 γgm./gm. body-weight, *BALB/C* × *C57* mice) produced characteristic convulsions within 2–4 min. The stages were: first hypersensitivity to auditory stimuli, followed by muscular twitching and vertical head tremor, clonus first appeared in the hind limbs and then became more generalized with jumping. Between convulsions the animal appeared flaccid with apparent paralysis of the fore and hind limbs. Clonic attacks were followed by rapid respiration, gasping, exophthalmos and death. With lower doses the severe clonic attacks did not occur and surviving animals were subdued for up to 90 min.

The LD_{50} at room temperature (20°) for pithecolobine injected intraperitoneally was 40 γgm./gm. body-weight, the LD_{50} was approximately halved at both 4° and at 36°. Doses of 12 γgm./gm. body-weight of pithecolobine reduced the quinalbarbitone sleeping time of mice by 20 per cent; higher doses (12–18 γgm./gm. body-weight) increased it by up to 100 per cent. The toxicity of pithecolobine for mice was approximately doubled by the doses of quinalbarbitone (450 γgm./gm. body-weight) used for sleeping-time determination. When mice were given pithecolobine intraperitoneally (14 γgm./gm. body-weight) the rectal temperature, normally 37° C., dropped to 33° C.; when given quinalbarbitone alone it dropped to 29° C. and when given both it dropped to 26° C. Intravenous pithecolobine reduced the arterial blood pressure in the anæsthetized cat (4 mgm./kgm.) and rat (1·5 mgm./kgm.) by about 20 per cent for 5 min., both before and after vagotomy. In the anæsthetized rat, hypotensive doses of pithecolobine caused a marked increase in the amplitude and decrease in the rate of respiration. Perfusion of the spinal cord of the frog[4] with pithecolobine (15 γgm./ml.) reduced or abolished the electrically stimulated reflex contractions of the femoral biceps.

The most striking peripheral effect of pithecolobine is its local anæsthetic activity. It is apparently twice as potent as cocaine in the frog lumbar plexus test for local anæsthetics[5]. The intradermal weal test on guinea pigs[5] showed that injection of 1–3 mgm. of pithecolobine caused local anæsthesia for up to 8 hr. Similar results have been obtained in man. Pithecolobine did not produce significant corneal anæsthesia[6] in guinea pigs or rabbits. Pithecolobine appeared to have a generalized depressant effect on isolated organ preparations; for example, on the electrically stimulated phrenic nerve diaphragm[7], on the contractions of the guinea pig ileum induced by acetylcholine, histamine, nicotine and barium, on the contractions of the rat fundus strip induced by 5-hydroxytryptamine[8], and on the Langendorff preparation of the isolated rabbit heart.

The nature of the convulsions caused by pithecolobine and its effects on respiration, sleeping-time, and possibly temperature, suggest that this substance has some action on the brain stem. These pharmacological actions of pithecolobine are of particular interest because of the very unusual structure[2] of this alkaloid. Chemical studies of the alkaloids of the shade tree are under way at the University College of the West Indies and we hope to investigate fully both the pharmacology and biochemistry of pure alkaloids from this source.

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Note added in proof. Further work suggests that the local anæsthesia produced by intradermal injections of pithecolobine is due to damage to nerve endings.

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Urea Synthesis in the Living Rat Brain

In previous communications from this laboratory, the synthesis of urea-^{14}C from arginine-^{14}C was reported to occur in the living rat brain[1,2]. In these experiments only the rat brains were analysed for their radioactive urea content, and the objection was raised that perhaps these levels did not indicate endogenous brain urea synthesis, but were merely a reflexion of blood ^{14}C-urea levels, especially since it is known that urea readily diffuses across the blood brain barrier. Experiments were therefore designed to show that the brain ^{14}C-urea levels were independent of blood ^{14}C-urea levels and that the brain had the capacity to synthesize urea from arginine. This communication describes such experiments.

Guanido-^{14}C labelled L-arginine-hydrochloric acid was obtained through the courtesy of the California Corporation for Biochemical Research before it was commercially available and was further purified by descending paper chromatography in two consecutive solvent systems. Solvent 1: pyridine/acetic acid/water (50 : 35 : 15). Solvent 2: ethanol/water/diethylamine (76 : 23 : 1). Identification was determined using the ninhydrin spray[3], and a modification of the Sakaguchi test[4] in conjunction with coincidence of radioautography. The R_F of the arginine in the above two solvent systems was 0·40 and 0·46 respectively. The purity of the arginine-^{14}C was verified by chromatography of the arginine eluate from the second solvent system in separate solvent systems of 2,6-lutidine/collidine/water/diethylamine (1 : 1 : 1 : 2 per cent v/v), propanol/acetic acid/water (75 : 5 : 20) and 2,4-lutidine/collidine/water/diethylamine (1 : 1 : 1 : 2 per cent v/v), propanol/acetic acid/water (75 : 5 : 20) and 2,4-lutidine/collidine/acetic acid/water (1 : 1 : 5 per cent v/v : 2).

A 90-day-old male albino rat (Sprague–Dawley strain) was lightly anæsthetized with diethyl ether and the exposed saphenous vein was cannulated with a heparinized, polyethylene cannula. 5 min. later an intracisternal injection of 0·4 mgm. of guanido-^{14}C labelled L-arginine-hydrochloric acid was administered using a micro-calibrated syringe (total activity 10 μc. = $7·3 \times 10^6$ counts/min.). At intervals following the injection, 0·5 ml. samples of blood were withdrawn from the vein and analysed for the specific activity of radioactive urea. 60 min. after the injection, the animal was killed by decapitation and the brain analysed for radioactive urea. As a control for possible injection of urea-^{14}C as a contaminant of the arginine preparation as well as a control for urea formation from arginine as a result of the procedures employed, a litter-mate rat was killed and to the isolated brain was added 0·4 mgm. of guanido-^{14}C labelled L-arginine-hydrochloric acid. The brain was treated as described here.

Following the withdrawal of the blood samples from the experimental animal, 5 ml. of 15 per cent trichloracetic acid (TCA) were added and the samples were homogenized. 8 mgm. of unlabelled carrier urea were then added to the homogenates; the samples were centrifuged and the supernatant solutions were decanted and saved. The TCA from these fractions was then extracted with carbon tetrachloride. The samples were then taken almost to dryness with a rotary evaporator and the insoluble dixanthydrol derivative of urea was prepared and recrystallized to constant specific activity. The brain was homogenized and urea was isolated from the acid-soluble fraction by ion-exchange column chromatography. A derivative was prepared as described here.

Analyses for radioactivity and urea content were performed in a manner reported previously[1].

The results presented in Table 1 show that the specific activity of the brain urea is at least thirty times as great as the highest blood urea specific activity. The maximum urea-^{14}C contamination as determined by the specific activity of urea in the control brain is 6×10^3 counts/min./mgm. urea. If the brain urea were derived only from exogenous sources and was transported to the brain via the blood, then the brain urea specific activity could not be higher than that of the blood[5]. The fact that it is many times greater than the blood level, at all intervals in which blood was collected, is proof of endogenous brain urea synthesis from arginine.

Table 1. Specific Activity of Blood* and Brain* Urea Samples

Sample	Time (min. after injection)	Specific activity of urea (counts/min./mgm. urea)
Blood 1	18	3×10^3
2	20	3×10^3
3	30	3×10^3
4	40	5×10^3
5	50	6×10^3
6	57	7×10^3
Brain	60	250×10^3

* The whole rat brain contains 0·5 mgm. urea, and 0·5 ml. rat blood contains 0·29 mgm. urea.

Note: Ratner *et al.* have shown recently in partially purified rat-brain proteins that arginase is present; this is verified by our *in vivo* work[6].

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Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain

Immunological studies of the cerebrospinal fluid have been confined mainly to the passage of antibodies from plasma into the cerebrospinal fluid[1]. However, the investigation of exchange between cerebrospinal fluid and blood has revealed that various electrolytes[2], dyes[3] and proteins[4] may find their way from cerebrospinal fluid into general circulation. Furthermore, some data[5] indicate that red blood cells cross cerebrospinal fluid-blood barrier. It seemed, therefore, advisable to approach the immunological sequences following the injection of heterologous erythrocytes into lateral ventricle of the brain.

Table 1. Peak Agglutinin and Hæmolysin Titre (log_2) in the Serum and Cerebrospinal Fluid (CSF) of Rabbits receiving Sheep Erythrocytes into the Lateral Ventricle of the Brain

Rabbit No.	Agglutinin				Hæmolysin			
	Before injection of sheep red cells		Day after the last injection of sheep red cells: 2 4 6 8 10 12*		Before injection of sheep red cells		Day after the last injection of sheep red cells: 2 4 6 8 10 12*	
	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
4	2	0	2 2 3 4 5 5	0	5	0	11 13 14 12 10 9	1
6	1	0	3 5 6 6 7 6	0	5	0	11 13 14 15 14 12	1
7	1	0	3 5 5 5 5 4	0	8	0	12 14 12 10 10 9	1
8	1	0	1 3 4 4 4 3	0	8	0	9 9 11 11 11 10	0

* Day when the rabbits were killed.

Young adult rabbits weighing 2·8–3 kgm. were used. A permanent cannula described by Feldberg and Sherwood[6] was implanted aseptically under pentobarbitone anæsthesia into the left lateral ventricle of the brain. The cannula was inserted at right angles to the bone, 7–8 mm. deep. The point of insertion lay 2 mm. lateral from the sagittal suture and 2 mm. behind the coronal suture. After the cannula was screwed into the skull and communication with the ventricular fluid was established, it was fixed to the bone by 'Texton' (S. S. White Co. of Great Britain, Ltd., London). Four days later the rabbits were treated for three successive days with a 5 per cent suspension (spectrophotometrically standardized) of washed sheep red cells. Two injections of 0·3 ml. each were performed daily, separated by a 1-hr. interval. The injections were all made into the lateral ventricle of the brain. After the last injection of antigen rabbits were bled every second day. The sera were heated at 56° C. for 20 min. and stored at −20° C. until titrated. Twelve days after the last injection the cerebrospinal fluid was withdrawn from the cisterna magna. The cerebrospinal fluid obtained was perfectly clear and not contaminated with red cells. Rabbit sera and cerebrospinal fluids were investigated for the presence of hæmolysin and agglutinin. The hæmolysin titration was essentially the same as described by Kabat and Mayer[7]. Hæmolytic activity was measured in 50 per cent units in the presence of five 50 per cent units of complement. Veronal buffered saline containing optimal concentration of magnesium and calcium ions was used for all washings and dilutions. The agglutinin content of sera and cerebrospinal fluids was determined by a semi-quantitative agglutination technique. Samples of rabbit sera and cerebrospinal fluids taken prior to the administration of sheep red cells were also investigated for the presence of hæmolysin and agglutinin.

Table 1 summarizes the results. It was found that cerebrospinal fluid, prior to the injection of antigen, did not exhibit hæmolysin and agglutinin activity, although natural antibodies were present in the serum. However, when the concentration of hæmolysin in the blood was sufficiently high it was possible to detect small amounts of these antibodies in the cerebrospinal fluid[8]. The injection of sheep erythrocytes into the cavity of rabbit brain was accompanied by an increase in antibody formation, the production of hæmolysin being much higher than that of agglutinin. Despite a high concentration of hæmolysin in the serum, only traces of that antibody were found in the cerebrospinal fluid. In a preliminary work it was noticed that rabbits which received only one intraventricular injection of sheep red cells manufactured specific antibodies at a very low level.

Bowsher[4] demonstrated that radioactive homologous serum protein, when injected into the subarachnoid space or ventricular cavities of the cat brain, was absorbed by different routes (leptomeningovascular, perineuro-lymphatic and/or ependymal). In rabbits we have also observed (unpublished experiments) that human γ-globulin inoculated into lateral ventricle may find its way to the general circulation, and provoke the appearance of specific precipitin in the serum. With rabbit serum taken 6 days after antigen injection a well-defined precipitin line was revealed by diffusion gel technique. The clearance of red cells from the cerebrospinal fluid, however, seems to have a separate mechanism. Essick[5] states that mesothelial cells may act as macrophages. Dupont *et al.*[9], using tagged red cells, have recently shown that the transfer of erythrocytes from cerebrospinal fluid to plasma is carried out by the mesothelial-lining cells of the subarachnoid space. These cells phagocytize erythrocytes and migrate towards the blood, thus causing slow appearance of radioactivity in the circulation. It may be supposed, therefore, that this slow removal of red cells from the cerebrospinal fluid to blood may act as a prolonged stimulus on antibody-producing apparatus, resulting in a significant increase of antibody in the serum. Finally, it seems that the intraventricular route of antigen administration offers a useful tool in the hands of an immunologist, and provides a new approach to the immuno-physiological examination of the central nervous system.

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Relationship between the Decrease in Urinary Excretion of Histamine and the Initiation of the Labour Process

As has been shown by Kahlson *et al.*, in rats fed on a histamine-free diet, the urinary excretion of histamine begins at about the fifteenth day of pregnancy and increases rapidly. The peak value occurs 1 or 2 days before birth, then falls steeply to the pre-pregnant level. The increase in histamine excretion depends on the number of young in the litter; the larger the number, the greater the increase in histamine excretion[1].

Investigations were made to demonstrate the relationship between the decrease in histamine excretion and the initiation of the labour process. Observations were made on white laboratory rats, fed on a normal diet. The method described in detail by McIntire, Roth and Shaw[2] was adapted to measure the amount of histamine in urine.

Table 1. Mean Daily Excretion of Histamine (μgm.)

	Control animals	Experimental	
Before pregnancy	50	50	
Days after mating 15	120	130	
20	880	750	
21	104	700	Administration of histamine
22	60	750	Administration of histamine
23	—	200	
24	—	60	
25	—	100	

Two days before the time of the expected parturition, that is, just before the onset of the period of low histamine excretion, 1·5 mgm. of histamine hydrochloride daily, in three doses, was injected subcutaneously during light ether anæsthesia. In this way the pretermal decrement in urinary histamine was prevented, and the high daily excretion (500–850 μgm.) was maintained artificially for two days.

It has been observed that, after the last injection of histamine, urinary excretion fell gradually during 1·5–2 days to about the pre-pregnant level. The labour process began about 30 hr. after decrease of the histamine-level. In this way the pregnancy was prolonged for two days. Some of the young in the litter from such a prolonged pregnancy were born dead; others showed after-birth weak respiratory movements, and died soon afterwards.

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RADIOBIOLOGY

Estimation of Time of Generation of Living Cells

The use of tritiated thymidine as a labelling agent has greatly facilitated examination of nuclear reproduction and cellular proliferation[1–4]. In such work, it has been customary to focus attention on the proliferation and fate of the cells that are labelled. Mendelsohn, however, has studied the fate of both labelled and unlabelled cells in tumours of mice and has used data obtained from both the labelled and unlabelled cell populations to derive the ratio of proliferative cells to total cells (the growth fraction)[5,6]. In the method presented here, applicable whenever a cohort of cells (that is, a fraction of the population) is labelled, the data for the labelled and unlabelled cells are analysed separately. In this way more information is obtained than when either only the labelled cells are studied or when data for the two cell populations are combined. The generation time of both labelled and unlabelled cells can be determined and compared, thus providing a method by which one aspect of toxicity of tritiated thymidine can be examined.

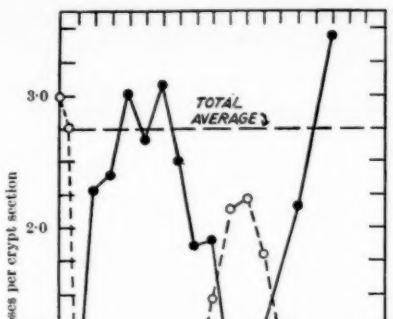

Fig. 1. Number of mitotic figures per crypt section as a function of time after injection of tritiated thymidine: dashed curve, unlabelled figures; solid line, labelled figures. 'Total average' represents the total number of mitotic figures per crypt section averaged over the 16-hr. observation period

The following experiment illustrates the use of this method in the determination of generation time and mitotic time of the labelled cells, and generation time of the unlabelled cells. CAF_1 mice, 93 days old, (weighing 25 gm.) received intraperitoneally 50 μc. of tritiated thymidine (360 mc./mM). Thereafter at intervals up to 16 hr., 1 or 2 animals were killed to obtain samples of the duodenum. Autoradiographs were made[7] of 3μ duodenal sections pre-stained by the Feulgen reaction. For each time of killing, the numbers of labelled and unlabelled mitotic figures (all stages) were counted in about 20 crypts of Lieberkühn (about 1,000 cells). The mean numbers of labelled (M_1) and unlabelled (M_u) mitotic figures per crypt section were calculated from these data and plotted as a function of time (Fig. 1).

Generation Time. In the case of the unlabelled cohort, the curve traced by M_u began at 3·0 mitoses per crypt section; at 2 hr. after injection the value was zero; the value began to increase again at 7 hr., and a peak value of 2·2 was reached at 11 hr. Thus a cycle was completed in 11 hr., which is an estimate of the generation time. If the duration had been measured between similar points (in the range $M_u = 1·5–0·5$) on the descending limbs of two successive cycles, it would have been about 12 hr. We therefore took the generation time to be 11·5 hr.

In the case of the labelled cohort, the curve traced by M_L began at zero and after passing through a peak of about 3 mitoses per crypt section (in agreement

with the initial value for the unlabelled cohort) returned to a minimum value at 11 hr. The generation time was therefore estimated as 11 hr., in good agreement with that for the unlabelled cohort. The estimate based on the time elapsing between successive M_L values of 3 (approximately the maximum) was 11·3 hr. We therefore took the generation time to be 11·2 hr. This result is in good agreement with our previously reported figure of 11·5 hr.[3].

For 2–9 hr. inclusive, the values of M_L (1·85–3·07, Fig. 1) had standard errors of 0·25–0·54. The full statistical analysis of these and further data will be discussed in a later report.

Mitotic Time. The mitotic time T of the labelled cells can be estimated as follows: $T = G\overline{I} = G\overline{N}/P$ where for the labelled cohort, G is generation time, $\overline{I}$ is mean mitotic index, $\overline{N}$ is the average number of cells in mitosis (per crypt section) throughout the generation cycle, and P is the number of proliferative cells (per crypt section).

The generation time of the labelled cells (G_L) was 11·5 hr. The average number of labelled cells in mitosis ($\overline{N}_L$) was determined by taking the average of 11 consecutive hourly determinations of labelled cells in mitosis (M_L) (Fig. 1); $\overline{N}_L = 1·91$. The number of labelled proliferative cells (P_L) was taken as the number of labelled cells (per crypt section) ½ hr. after the injection of tritiated thymidine, when the initial labelling was maximal and no labelled cells had gone through mitosis; $P_L = 15·5$.

From these data it follows that T (for labelled cohort) $= 11·2 \times 1·91/15·5 = 1·38$ hr.

The same procedure can be used to obtain the mitotic time of the unlabelled cells when the number of unlabelled proliferative cells can be accurately assessed. As yet we have not established the number of unlabelled proliferative cells at the necessary time-intervals with sufficient accuracy to calculate the mitotic time of the unlabelled cells.

The agreement between the results for the labelled and unlabelled cells indicates that the dose of tritiated thymidine used (50 μc. per animal) did not affect the generation time. It should be noted that this result applies only to that part of the first generation-cycle that has been studied. It remains to be determined whether or not the duration of subsequent cycles is affected.

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Leukæmia in the *AkR* Mouse after X-Irradiation *in utero*

It is known that the incidence of lymphoid leukæmia in mice is influenced by X-irradiation of the adult[1]. In strains where the spontaneous incidence of the disease is low, it is increased; but it may be decreased when the incidence is already high[2]. In the high-leukæmia strain *AkR* mouse, it has been shown that, after doses of X-rays of between 50 and 500 r. to the new-born animal, there is a reduction of the usually high incidence of leukæmia, slightly among females but more markedly among males even at the lowest doses[3].

The work reported here is part of an investigation of leukæmia after small doses of X-rays to the mouse fœtus. Retrospective and prospective studies of leukæmia after diagnostic X-rays to the fœtus in man have given conflicting results[4]. The present experiment was designed to determine whether there is an alteration of incidence of leukæmia or latent period in the high-leukæmic strain *AkR* mouse after small doses of X-rays to the fœtus.

AkR female mice were mated to *AkR* males which were their respective litter mates and then put into one of three treatment groups after the appearance of copulation plugs: (1) those to be sham irradiated; (2) those to receive 5 rads at the level of the gravid uterus on day 7½ and again on day 14½ of pregnancy; (3) those to receive 25 rads at the level of the gravid uterus on day 7½ and again on day 14½ of pregnancy.

The X-ray source was a 'Resomax' operating at 300 kV. and 13 m.amp. (filtration 0·1 mm. tin + 0·5 mm. copper + 1·0 mm. aluminium). The dose-rate was 5 rads per min., at a treatment distance of 140 cm. The females were allowed to rear their litters, which after weaning at 6 weeks were kept 6 per cage (2 from each treatment group) under identical conditions, males and females separately.

All animals were observed and eventually killed when moribund. Diagnosis of leukæmia was verified by autopsy, blood count and blood and bone marrow smears. Any doubtful cases were investigated by bioassay. The histological appearance of the leukæmia occurring in all groups conformed to that of the spontaneous lymphoid leukæmia characteristic of this strain.

A total of 353 animals was observed, 178 males and 175 females, progeny alive at the time of the first case of leukæmia at 180 days and deemed to be at risk. Table 1 contains a summary of results obtained after all the animals had died. No real difference in incidence of leukæmia can be shown in the female groups. In the males, however, the group treated with 50 rads shows a lower incidence of leukæmia than that of the control or of the 10-rad groups.

The mean time to death from leukæmia in the female groups seems to be unaffected by irradiation. In the males, however, the mean time in the 50-rad group is longer than in the 10-rad or control groups. (An analysis of variance shows this lengthening to be significant at the 10 per cent level.) The times to death from leukæmia for individuals in each group show a normal distribution against the logarithm of the time. The tail of the distribution curve suggests that all animals would have succumbed to leukæmia if death from other causes had not intervened. In order to arrive at a more realistic mean for time to death from leukæmia in each group, allowance should be made for animals dying from other causes. The individual times of death for such animals have been

Table 1

Total dose (rads)	No. of animals at risk		Per cent leukæmia		Corrected mean time to death from leukæmia (days)	
	Males	Females	Males	Females	Males	Females
0	68	62	86·8	96·7	340 (333) ± 11	288 (288) ± 10
10	56	58	85·9	89·6	341 (333) ± 12	292 (290) ± 10
50	54	55	72·2	90·9	378 (362) ± 15	297 (294) ± 10

adjusted on the basis of a preliminary estimate of the distribution. The correction involves an addition of small magnitude which gives the probable time of death from leukæmia if the other cause had not operated. Corrected means are shown in the right-hand column of Table 1 with uncorrected means in parentheses.

It would appear that, after the doses of X-rays used, there is no evidence of either increase in leukæmia incidence or of accelerated disease in either males or females irradiated *in utero*. On the contrary, in males after 50 rads there may be a decrease in incidence of leukæmia with an increased mean time to death from leukæmia.

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HÆMATOLOGY

Hæmagglutinins from *Clerodendrum viscosum* Vent

The capacity of certain plant proteins closely to simulate the action of various human blood group specific agglutinins is well known; the subject has been reviewed by me[1]. Almost all strong blood-group-specific plant agglutinins have been obtained from the genus Leguminosae; recently a good anti-*A* (anti-A_1) was found in the seeds of *Hyptis sauveolens* Poit of the genus Labiatiae[2]. Examination of other non-leguminous plants has now revealed specific agglutinins in the fruit pulp of *Clerodendrum viscosum* Vent (syn. : *C. petasites* Lour; *C. infortunatum* Cook *et al.*, non-Gaert and non-Linn), of the natural order Verbenaceae, a gregarious shrub commonly found throughout India, Burma, Ceylon and the Andaman Islands.

The fruit is about the size of a large garden pea. It has a relatively big seed which contains weak non-specific agglutinins. The fruit pulp was carefully separated from the seed, ground in three times its volume of physiological saline, and filtered. The filtrate, when tested for avidity by the tile method, and titrated by the conventional tube method, was found to agglutinate red cell suspensions of various *ABO* blood-groups and sub-groups in the following order: *O* (strongest), A_2, A_2B, *B*, A_1, A_1B; *H*-negative cells were not agglutinated. The reactions were not influenced by the *Le*^{a} or *Le*^{b} status of the erythrocytes. Thus the agglutinins of *C. viscosum* are anti-*H* or anti-*O*.

The agglutinin is inhibited, but not completely neutralized, by a 1/1,000 (w/v) aqueous solution of purified *H*-substance; it is not inhibited by identical concentrations of similarly purified *A*, *B* or *Le*^{a} substances. Similarly, it is inhibited, but not neutralized, by secretor salivas; it is not inhibited by non-secretor salivas. The agglutinin is inhibited (two tubes) by 2 per cent salicin; it is not inhibited by 2 per cent L-fucose. It thus belongs to the little-understood group of anti-*H* agglutinins, which are not inhibited by L-fucose, a sugar which is an important structural determinant of *H*-specificity[3].

The *Clerodendrum viscosum* agglutinin would be useful for making the A_1: A_2 distinction, particularly when used in parallel with the anti-A_1 agglutinin from *Dolichos biflorus* seeds. It is not recommended for general use in making the secretor : non-secretor distinction, for which the anti-*H* agglutinin from *Ulex europaeus* seeds would be more reliable. However, it could be used for this purpose by those familiar with its properties, particularly in countries such as India, in which other plant sources of anti-*H* are not readily available. It might also be useful, as an addition to the heterogeneous ranks of anti-*H* reagents, in research on the *H*-character or characters of erythrocytes and secretions.

I am indebted to Prof. W. T. J. Morgan, for the blood-group substances and sugars; to V. B. Kumar, for collecting the plants and for technical assistance; and to the Botanical Survey of India, Poona, for the identification.

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Hæmoglobin *P* in a Family of Southern Italian Extraction

The larger part of abnormal hæmoglobins described in world literature has been observed, in sporadic form, among the people of Italy. As a matter of fact, Silvestroni and Bianco *et al.* have identified[1–3] not only many cases of hæmoglobin *S*, the presence of which in Italy was known for some time, but also cases of hæmoglobins *L*, *D*, *G*, *K*, *H* and A_2 abundant in non-microcythæmic (or thalassæmic) subjects.

Now another abnormal hæmoglobin, *P*, has been found in four members of an Italian family originating from Calabria. During work on the electrophoresis of the hæmoglobin of microcythæmics, a carrier of microcythæmia (or thalassæmia minima), F. Bettina, aged seventeen, was first found, and later three of her brothers (F. Francesco, aged

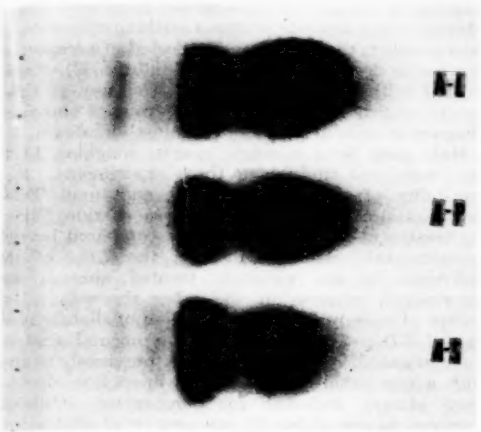

Fig. 1. Paper electrophoresis of hæmoglobins *A–L*, *A–P* and *A–S*, glycine buffer *p*H 8·8, ionic strength 0·04. Stained with bromophenol blue. Mobility of the hæmoglobin slow fraction identical in the three samples

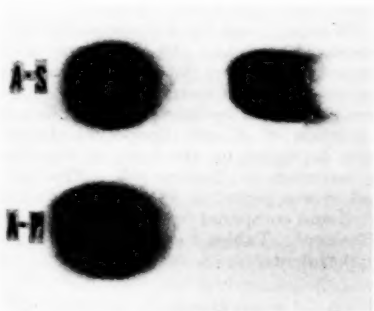

Fig. 2. Electrophoresis in agar gel of hæmoglobins *A–S* and *A–P*. Citrate buffer *p*H 6·2, ionic strength 0·05. Stained with bromophenol blue. Complete lack of separation of hæmoglobin *P* from hæmoglobin A_1

twenty-three ; F. Giuseppina, aged twenty-seven ; F. Maria, aged fifteen) and their grandfather on the mother's side (T. Francesco, aged seventy) were found carriers of a slow hæmoglobin ; all four were enjoying good health and their hæmatological pattern was perfectly normal, without presenting any character reflecting the simultaneous presence of microcythæmia, the fraction of hæmoglobin *a–r* being below 2 per cent and that of hæmoglobin A_2 between 2 and 2·60 per cent, whereas in the microcythæmic member it was 4·50 per cent. The slow hæmoglobin was present in all four subjects to the extent of about 50 per cent.

An investigation to determine the characters of this abnormal hæmoglobin has produced the following results :

(1) In paper electrophoresis at *p*H 8·6, the mobility of the slow hæmoglobinic fraction was equal to that of hæmoglobins *S*, *D* and *L* (Fig. 1).

(2) Even on starch-block and on starch-gel electrophoresis at *p*H 8·6 the slow hæmoglobin separated from hæmoglobin A_1 with the same mobility as that of hæmoglobin *S*.

(3) In paper electrophoresis and starch gel at *p*H 6·2–6·5 hæmoglobin A_1 and the slow hæmoglobin did not separate, and the hæmoglobin spot had the same aspect as that of hæmoglobin A_1 only.

(4) Even in agar gel at *p*H 6·2 the slow hæmoglobin did not separate from hæmoglobin A_1 (Fig. 2), contrary to hæmoglobins *S* and *L*, and like hæmoglobin *D*.

(5) In column chromatography using 'Amberlite *IRC*–50' at *p*H 6, the slow fraction did not separate from hæmoglobin A_1; the hæmoglobin band was, however, much wider than that of hæmoglobin A_1 alone, extending from this to the band of hæmoglobins A_2, *G* and *S* (Fig. 3).

(6) The ultra-violet spectrum of the slow hæmoglobin, isolated after starch block electrophoresis, was quite normal and identical with that of hæmoglobin A_1 of the same subject.

(7) In alkaline denaturation test, the isolated slow hæmoglobin behaved as hæmoglobin A_1 and denatured completely within 60 min.

These characters of the slow hæmoglobin examined correspond clearly with those described for hæmoglobin *P* [4–6]; in particular, the more specific characters of this hæmoglobin and which more clearly distinguish it from the other slow hæmoglobins (failure to separate from hæmoglobin A_1 in agar gel and during chromatography at acid *p*H) are certainly present in the hæmoglobin under examination.

The only discordant point appears to be that of electrophoretic mobility at alkaline *p*H, which in the initial observation of Schneider and Haggard[4,5] was defined as equal to that of hæmoglobin *L*, even though it has been observed in analyses between glass plates or electrophoresis of less than 18 hr. duration, that "its pattern was indistinguishable from that of hæmoglobin *A–S*". In previous analyses of other slow hæmoglobins, Silvestroni and Bianco *et al.*[3] have been able to demonstrate that the mobility of a slow hæmoglobin fraction is, within certain limits, dependent on its concentration in the hæmoglobin mixture, that is, the lower the aforesaid concentration, the more it decreases. This fact has been fully confirmed also for the present slow hæmoglobin, and comparisons of electrophoretic mobility have therefore always been made with mixtures of hæmoglobins containing the same percentage of slow hæmoglobin.

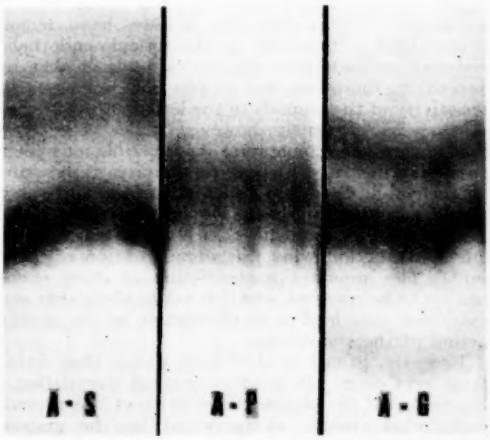

Fig. 3. Chromatography on columns of 'Amberlite *IRC*-50' of hæmoglobin *A–S*, *A–P* and *A–G*. Lack of separation of hæmoglobin *P* from A_1 and formation of a diffused hæmoglobin band

Therefore, as the difference of mobility is very slight and also so easily affected by the 'concentration factor', it can be stated, in conclusion, that it cannot have any value.

Hence the hæmoglobin described here is certainly a new case of hæmoglobin *P*: it seems that this is the fourth observation described in the literature after that of Schneider and Haggard[4,5], Dherte *et al.*[6] and Lambotte-Legrand *et al.*[7]; the second in which hæmoglobin *P* is present without any association of other slow hæmoglobins (*S*); and it is the first case of hæmoglobin *P* found in Italy.

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PATHOLOGY

Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the Reticulo-endothelial System

It has generally been assumed that resistance of animals to the pyrogenic and lethal effects of bacterial endotoxin is largely dependent on the phagocytic capacity of the reticulo-endothelial system. Thus it has been shown that stimulation of the reticulo-endothelial system with non-lethal doses of bacterial lipopolysaccharide or endotoxin results in protection of the animals against a subsequent lethal dose of endotoxin[1]. Again, numerous workers have demonstrated that a 'blockade' of the reticulo-endothelial system by colloidal particles, for example saccharated iron oxide, thorotrast and trypan blue, increases the sensitivity of the animals to the lethal effects of these substances[2]. Recently, however, it has been shown that in certain circumstances it is possible for mice to possess an increased phagocytic activity, as measured by their ability to clear colloidal carbon, and yet be highly sensitive to the effects of endotoxin. This can be seen during the course of an intracellular infection[3], or following the administration of zymosan[4]. The apparent contradiction of these results has yet to be resolved, and it is not unlikely that such resolution may lead to an elucidation of the mode of action of the endotoxins.

Recently, Stuart *et al.*[5,6] have shown that certain fatty acid esters can produce marked stimulation or depression of the phagocytic activity of the reticulo-endothelial system, as measured by the granulopectic test[7]. Results of histological studies (to be reported elsewhere) have shown that glycerol trioleate (triolein) produces a marked increase in the activity of phagocytic cells of the liver and spleen, whereas ethyl stearate causes a striking reduction in their number; these effects are reflected in a respective increase or decrease of phagocytic indices (*K*) of mice treated with these substances. The use of these agents might help to clarify the role of the fixed phagocytic cells in protection against endotoxin.

Male mice from a single source, weighing 15–20 gm., were used throughout these experiments. Susceptibility of animals to a single endotoxin (Difco *Escherichia coli* 'O'111: *B*4 lipopolysaccharide) following treatment with the lipids was compared by two experimental systems. In one, the LD_{50} of the endotoxin in the variously treated animals was determined, whereas in the other the survival of groups of mice receiving an injection of slightly more than 1 LD_{50} of the endotoxin (for normal animals) was estimated. Groups of animals, previously treated with a non-lethal dose of *E. coli* lipopolysaccharide, were always included for comparison. Colloidal suspensions containing 20 per cent (v/v) triolein and 20 per cent (w/v) ethyl stearate were prepared as described previously[5]; the lipopolysaccharide was dissolved in isotonic saline in a concentration of 200 μgm./ml. The following dosage schedule was used throughout the experiments: triolein, 0·05 ml./20 gm. mouse (11·5 mgm.), ethyl stearate, 0·1 ml./20 gm. mouse (20 mgm.) and lipopolysaccharide 0·1 ml./20 gm. mouse (20 μgm.). All injections were given intravenously. 24 hr. later the mice, including appropriate groups of normal animals of the same age, were challenged by intraperitoneal injection of a saline solution of *E. coli* lipopolysaccharide. The dose used depended on the form of the experiment and is recorded in Tables 1–3. The mice were observed over a period of 48 hr.; LD_{50} values were calculated and compared by the method of Litchfield and Wilcoxon[8]. Tables 1 and 2 record the results of these experiments.

Table 1. LD_{50} of *E. coli* Lipopolysaccharide in Mice treated with Lipopolysaccharide and Simple Lipids

Treatment	Survival-rates following endotoxin doses of (in μgm.)					LD_{50} (μgm.) with 19/20 confidence limits
	500	400	300	250	100	
Normal	1/10	2/10	6/10	8/10	10/10	325 (276–357)
Lipopoly-saccharide	5/8	8/10	7/9	9/10	9/9	630 (437–908)
Triolein	1/10	1/10	1/10	4/10	7/10	175 (132–234)
Ethyl stearate	3/10	6/9	8/10	8/10	9/10	445 (326–615)

Survival-rate = No. alive/No. tested.

Table 2. Survival of Animals treated with Lipid or Lipopolysaccharide following Challenge with 400 μgm. *E. coli* Lipopolysaccharide

Treatment	Survivors/tested	Survival (per cent)
Normal	19/40	47·5
Lipopolysaccharide	37/40	92·5
Triolein	6/40	15
Ethyl stearate	21/40	52·5

Table 3. Phagocytic Indices of Mice 24 hr. after Treatment with Lipids and *E. coli* Lipopolysaccharide

Treatment	Animals	Mean K* values	Mean α* values
Normal	10	0·037	5·4
Lipopolysaccharide	10	0·096	7·0
Triolein	10	0·105	7·7
Ethyl stearate	10	0·0125	3·4

* K and α values were determined at a dosage of 8 mgm./100 gm.

The granulopectic activity of mice 24 hr. after injection of the lipids or the *E. coli* lipopolysaccharide was determined by the technique of Halpern *et al.*[7]. The phagocytic index (K) and the corrected phagocytic index (α) were determined at a dosage-level of carbon of 8 mgm./100 gm. Results of these investigations are summarized in Table 3.

Although triolein stimulates as good, or better, phagocytic activity as does lipopolysaccharide treatment, these results demonstrate that this lipid renders the mice significantly more susceptible to endotoxic activity. Again, contrary to the findings with 'blockaded' animals, the removal of phagocytes following treatment with ethyl stearate does not appreciably enhance susceptibility to endotoxin. It is arguable that the endotoxin may be taken up by phagocytic cells other than those of the liver and spleen, although it should be noted that in normal animals these organs together remove more than 80 per cent of the endotoxin[9].

The increased susceptibility of animals following triolein is not so great as that recorded following *BCG* infection or zymosan treatment. This difference may be related to the finding that, after either *BCG* infection or administration of zymosan, a marked granulomatous response occurs, whereas the simple lipid apparently exerts its action only on those sessile phagocytic cells which are in contact with the blood. At this stage the results suggest that endotoxin activity is dependent on the rapidity of uptake by certain cells, particularly those of the reticulo-endothelial system. Certainly it seems that the more phagocytes present in the liver and spleen the more sensitive are animals to endotoxin; on the other hand, decrease in cell numbers appears to have, if anything, a slightly beneficial effect on the resistance of the animals.

Lipopolysaccharide treatment may involve not only an increase in phagocytic cells but also perhaps stimulation of intracellular mechanisms of detoxification. Nevertheless, the influence of endotoxin-neutralizing factors in sera cannot be overlooked in assessing the protective activity of these substances[10].

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Non-Specific Immunity in Pneumococcal Infection of Mice

The development of early, non-specific protection against experimental infections with Gram-negative micro-organisms has been observed following injection of bacterial endotoxin, lipopolysaccharide and lipid A [1]. Reports of similar activities of these substances against infections with Gram-positive organisms are less numerous and have been substantiated only in the case of infection with *Staphylococcus pyogenes* or *Mycobacterium fortuitum*[2]. In an attempt to assess the role of phagocytosis by cells of the liver and spleen in this non-specific protection, we have utilized certain fatty acid esters which are capable of profoundly increasing or reducing the activity of these cells[3]. One of the experimental models selected for this work has been infection with *Streptococcus pneumoniae* in mice. Infections with this organism have usually been carried out by the intraperitoneal route and are highly lethal, unless the infection is modified by the classical means of active or passive immunization. In an attempt to demonstrate other, perhaps less potent, protective mechanisms, we felt that a less-virulent type of infection might be more appropriate. This has been achieved by using a strain of *Strep. pneumoniae* type 8, preparing the challenge inoculum in 0·1 M phosphate buffer at pH 7·4 and injecting the chosen dose of organisms by the intravenous route. Details of this technique will be published fully elsewhere.

Male mice derived from a single source and weighing 15–20 gm. were used. The effects of glycerol trioleate (triolein) and ethyl stearate treatment on the infection were compared with those of non-lethal doses of *E. coli* 'O'111 : *B*4 lipopolysaccharide (Difco). The method of preparing the lipid suspensions has been described previously[3]. The following doses of the substances were given to individual groups of mice by the intravenous route: triolein, 0·05 ml. of 20 per cent (v/v) suspension (11·5 mgm.), ethyl stearate, 0·1 ml. of 20 per cent (w/v) suspension (20 mgm.) and *E. coli* lipopolysaccharide, 0·1 ml. of 200 μgm./ml. solution (20 μgm.). 24 hr. later all the mice, including appropriate groups of normal animals, were injected intravenously with *Strep. pneumoniae* derived from an over-night culture in horse digest broth, and diluted appropriately in phosphate buffer. The number of organisms in the challenge dose was determined by viable count using the Miles and Misra technique[4]. Injections of the lipids and lipopolysaccharides were repeated 24 hr. later. The mice were observed for seven days and time to death within the groups was recorded. In Tables 1 and 2 are summarized the results of two experiments, one in which a constant challenge dose (about 4×10^4 organisms) was used, the other in which three challenge doses differing by ten-fold dilutions were given to various groups of animals.

It has been shown (preceding communication) that mice treated with lipopolysaccharide and triolein have a significantly higher phagocytic activity than normal when measured by the granulopectic technique[5]. Conversely, a marked suppression of phagocytic activity occurs following administration of ethyl stearate. These findings may be attributed to a marked increase or diminution of the hepatic and splenic phagocytes (Stuart, A. E., unpublished work). The results described here suggest that phagocytic reduction does not significantly affect the survival or time to death of the mice. However, a striking degree of

Table 1. Survival of Mice treated with Lipids and Lipopolysaccharide following Challenge with *Strep. pneumoniae* (4×10^4 organisms)

Treatment	No. survivors / No. tested	Survivors (per cent)	Mean time to death (hr.)
Normal	2/20	10	56
Triolein	6/20	30	73
Ethyl stearate	1/18	6	66
Lipopolysaccharide	18/20	90	108

Table 2. Survival of Mice treated with Lipids and Lipopolysaccharide receiving Graded Doses of *Strep. pneumoniae*

Treatment	Survivors/tested receiving challenge doses of: $2{\cdot}5 \times 10^3$ organisms	$2{\cdot}5 \times 10^4$ organisms	$2{\cdot}5 \times 10^5$ organisms
Normal	0/10	1/10	9/10
Triolein	3/10	4/10	9/10
Ethyl stearate	0/10	1/10	6/10
Lipopolysaccharide	7/10	10/10	10/10

protection against infection can be achieved with lipopolysaccharide, and to a much less extent with triolein. There seems no doubt of the non-specific nature of this enhanced protection, which develops within 24 hr. This protection cannot be related to the properdin system, which has been shown to be effective only against Gram-negative organisms (ref. 1, *b*).

The difference in protection achieved with lipopolysaccharide and triolein is of particular interest. There is no observable difference in the ability of animals to clear colloidal carbon from the blood following treatment with either of these substances. It is generally assumed that the granulopectic test is a measure of an animal's ability to clear microorganisms from the blood as well[6]. This is substantiated by an experiment, the results of which are recorded in Table 3, in which the removal of viable pneumococci from the blood of animals was examined. Groups of mice treated with lipopolysaccharide or triolein were injected intravenously with approximately 1×10^6 organisms (derived from an overnight culture, as above) suspended in phosphate buffer. Immediately after injection and at 6-min. intervals thereafter up to 18 min., blood samples were removed from the retro-orbital venous plexus; ten-fold dilutions of the sample were prepared in phosphate buffer and viable counts were determined by the Miles and Misra method[4]. Logarithms of the counts plotted against time yielded approximately linear curves, and it was thus possible to express the rate of removal of organisms (K) in terms of the slope of the line.

The results suggest that the differences in effects observed with the lipopolysaccharide and glycerol trioleate cannot be explained in terms of rate of removal of the organisms from the blood. Rather it seems that the lipopolysaccharide treatment stimulates some factor which causes more rapid death of the organisms following their removal. This may be either a serum factor (non-specific opsonin) or an enhanced intracellular activity of the phagocytes stimulated by the lipopolysaccharide. Preliminary experiments suggest that opsonization *per se* may have no immediate influence in non-specific protection against the pneumococcal infection.

Table 3. Rate of Removal of Viable Pneumococci (K) in Mice treated with Lipid or Lipopolysaccharide

Treatment	Mean values of K	KT/KN
Normal	0·013	
Triolein	0·031	2·48
Lipopolysaccharide	0·035	2·68

KT/KN = Ratio of K values of treated to normal animals.

One of us (G. N. C.) gratefully acknowledges the receipt of a Wellcome Research travel grant. The work of the other (A. E. S.) has been supported by a research fellowship awarded by the Scottish Hospitals Endowments Research Trust. Our thanks are also due to Prof. R. Cruickshank and G. L. Montgomery for their encouragement and advice.

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Increase in Specific Bactericidal Antibodies after Administration of Endotoxin

Many non-specific immune phenomena have been described in which animals became resistant to experimental infection following administration of antigenically unrelated materials. None has attracted more interest than the temporary state of enhanced resistance to infection with Gram-negative bacteria which develops in mice within hours after administration of antigenically unrelated bacterial endotoxins[1]. Among the many effects of endotoxins on the host is increased activity of the reticulo-endothelial system as shown by carbon clearance[2] and by removal of phosphorus-32 labelled endotoxin[3] or bacteria[4]. In addition, changes in the serum of endotoxin-treated animals have been reported, notably increased levels of properdin[5], bactericidins[6] and opsonins[7,8]. Passive transfer of opsonic capacity has also been demonstrated[4]. It is evident that these alterations in the host, individually or collectively, may contribute significantly to survival. Special emphasis has been placed on the non-specific nature of the humoral factors involved, because there has been abundant confirmation of activity against bacterial strains antigenically unrelated to the endotoxin used for stimulation. Cross-reacting antibodies[9] or a common antigen associated with the lipid complex of endotoxins[10] have therefore been suggested to account for these observations.

The serum bactericidal reaction against Gram-negative bacteria has been shown to depend on specific antibody, complement and divalent cations. We have developed a method for the determination of antibody based on this reaction[11]. Serum accessory factors, such as complement, are supplied in excess. The level of specific antibody in the serum under test is measured by the extent to which it restores bactericidal activity to absorbed human serum. In normal serum this method detects antibody against Gram-negative bacteria in amounts considerably less than

Table 1. BACTERICIDAL ACTIVITY OF MOUSE SERUM FOR *Salm. typhi* 0901

Serum (ml.)	Bacterial survival in serum from Normal mice (per cent)	Endotoxin-treated mice* (per cent)
0·01	7	3
0·0066	59	3
0·005	72	23
0·004	87	46
0·0033	95	68

The bacterial inoculum was 10^6 cells.

* 24 hr. following 50 μgm. *Sh. shigae* endotoxin i.v. The quantity of serum giving 50 per cent killing was 0·007 ml. in the case of normal mice and 0·004 ml. for the endotoxin-treated mice.

Table 2. SPECIFICITY OF THE BACTERICIDAL ANTIBODY OF ENDOTOXIN-TREATED MICE

Treatment of mice	Relative bactericidal activity of serum*			
	Unabsorbed		After absorption with heat-killed *Salm. typhi* 0901	
	Salm. typhi 0901	*Esch. coli* 0127	*Salm. typhi* 0901	*Esch. coli* 0127
None	1·0	1·0	0	1·0
50 μgm. *Sh. shigae* endotoxin i.v. 24 hr. previously	1·7	2·5	0	2·3

* Supplemented with absorbed human serum as a source of complement.

those required for agglutination of bacteria or antigen-coated red cells, namely, 0·001 μgm. antibody nitrogen in the case of antiserum to *Salmonella typhi*. The quantity of serum which gives 50 per cent bacterial killing is determined, with satisfactory reproducibility, by plotting log serum dose against probits of bacterial survivors. Our work has dealt predominantly with the mouse, because non-specific resistance has been investigated almost exclusively in this species. Mouse serum is not bactericidal, because its complement is not functional in this system; supplementation with heterologous complement is therefore essential for the assay. In our tests pooled normal human serum, from which antibody had been absorbed by heat-killed bacteria, served as the source of serum accessory factors.

With this method we have found that after administration of endotoxin to mice, in amounts which elicit so-called non-specific immunity, there is an increase in serum bactericidal activity against several antigenically unrelated strains of Gram-negative bacteria. For example, within 3 hr. after administration of 50 μgm./mouse of a *Shigella shigae* endotoxin, increased bactericidal activity for other bacterial strains was discernible which persisted for more than 48 hr.; by 96 hr. the level returned to normal. Table 1 shows the level of this increased bactericidal activity at 24 hr. Absorption of the serum with one Gram-negative serotype removed bactericidal activity for that strain, whereas the increased serum bactericidal activity against strains of other genera was retained. An illustrative experiment is summarized in Table 2. Thus the factors in serum responsible for the elevation of bactericidal effect not only have the functional characteristics of antibodies, but also are individually absorbable as would be expected of specific antibodies.

Additional evidence for the antibody-like character of the bactericidin was also obtained. Serum from endotoxin-treated animals, after absorption with heat-killed *Salm. typhi* 0901, still possessed increased bactericidal activity for *Escherichia coli* 0127. This absorbed serum, added as a supplement to normal mouse serum in a bactericidal test against *Salm. typhi* 0901, did not increase the bactericidal activity of normal mouse serum. Thus the increased bactericidal activity is due to specific antibody rather than to some non-specific factors.

The rise in bactericidal titre after administration of endotoxin is assumed to be due to a transitory effect of endotoxin on antibody-forming cells. This may involve either release of preformed antibodies or a temporary increase in the capacity of these cells to produce antibody. These results point to a temporary elevation of a whole array of specific antibodies rather than the appearance of non-specific or cross-reacting bacterial antibodies. Experiments are in progress to determine whether the reported increase in opsonic factors in mice, following the administration of endotoxin, involves the same specificities.

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BIOLOGY

A Natural Population of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda: Hymenolepididae), in a Colony of *DBA*/1 Mice

DURING work on metabolism of the dwarf tapeworm of mice (*Hymenolepis nana*), a natural infection with this parasite was found in a local colony of *DBA*/1 mice (Curd's Caviary, Downey, California). Of 500 mice from this colony examined over a two-year period, 320 were infected with an average of 35 worms per mouse, varying from a single 12-in. worm to one or two thousand extremely small ones (demonstrating a 'crowding effect'). Among more than 12,000 worms recovered, approximately 5 per cent showed one of the following malformations or growth disturbances: (1) Partial twinning (Fig. 1*A*): forked posterior strobila; (2) lateral branching (Fig. 1*B*): new tissue outgrowth with varying degrees of differentiation; (3) dedifferentiation (Fig. 1*D*): groups of segments appearing as undifferentiated, vacuolated granular regions; (4) segment reversal: reversal of normal antero-posterior sequence of proglottides (Fig. 1*B*); (5) combination: dedifferentiation plus branch outgrowth (Fig. 1*C*); branch formation with segment reversal (Fig. 1*B*); multiple branching (Fig. 1*E*).

Cestode abnormalities reviewed by Foster, Faust, Clapham, Kuntz and Dollfus and others[1–7] include fenestration of groups of segments, fusion of strobilar

loops, doubling and inversion of organs and other developmental irregularities, bud formation and dichotomy, spiral strobilization and polyradiate forms with additional suckers and marginal ridges. No one, however, has recorded recovery of numerous abnormalities from a single host population over a sustained period. The following circumstances are of added interest: (1) all aberrant worms were from a single commercial stock of mice, derived from the *DBA*/1 strain; (2) these hosts were maintained with a colony of Swiss Webster mice, with both groups showing a similar rate of infection, approximately 70 per cent, by *H. nana*. No branching was found among thousands of *H. nana* examined during a five-year period taken from 700 Swiss white mice from this colony. Nor were abnormal worms recovered from naturally infected mice from several commercial sources or from original Jackson Memorial Laboratory *DBA*/1 mice. Among infected Curd strain *DBA*/1 mice, approximately 60 per cent harboured abnormal worms, representing 800 or 5 per cent of the total recovered.

The commonest abnormality was a small nub or outgrowth of tissue showing considerable variation in length, orientation and degree of maturation. Less common were granulation and vacuolization, an apparent dedifferentiation or tissue destruction, bifurcation of posterior segments, and segment reversal. Multiple branching with varying degrees of maturation emphasize a capacity to bud or branch existing throughout the worm. Gravid branches were chiefly confined to twinned posterior ends. Abnormal worms were generally small, 3 cm. or less in length.

These malformations appear to be an interference with the normal pattern of differentiation and growth. New tissue development, usually confined to the neck area in tapeworms, appeared anywhere along the strobila, often accompanied by breakdown of organized regions. Subsequent differentiation and growth were delayed and abnormal. Branches usually showed more fully developed segments at branch-strobila junctions, the growing tip (equivalent to the neck of a normal worm) being carried out with the branch. No normal scolex was found, though neck-like, rounded branch ends often occurred (Fig. 1*B*, *C*). Twinned posterior ends formed by division of the terminal proglottides. This was followed by growth and maturation of the separate chains, their posterior portions therefore being most developed except for a terminal nub on the branch. Lateral branches probably formed similarly, but with sharply differing growth-rates for the two portions of the strobila.

These findings are of interest in terms of cestode growth patterns, antero-posterior differentiation and general growth control factors. Laboratory establishment of such an abnormal strain was therefore attempted. In three experiments, eggs cut from abnormal, gravid branches were fed in 0·1 ml. tap-water by pipette to six young non-infected *DBA*/1 mice. Gravid worms recovered were fed to normal white and *DBA*/1 mice and similarly passed through six generations of each host strain. No branched worms were recovered from any of these infections. In repeated feedings with intact abnormal worms to mice of Swiss, *DBA*/1 (Jackson Laboratory), and Heston/*A* (University of California Cancer Laboratory) strains, again only normal worms developed. Laboratory infection of Curd *DBA*/1 stock could not be attempted owing to absence of parasite-free animals from this source.

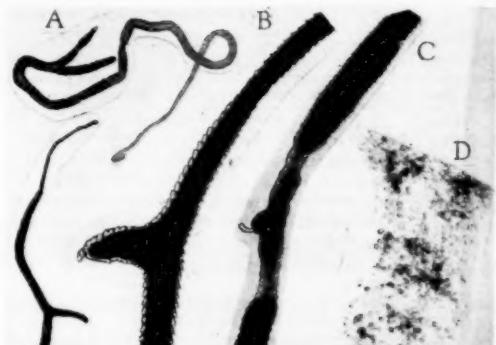

Fig. 1. Various expressions of tapeworm abnormality recovered from local strain of *DBA*/1 mice. *A*, Terminal twinning of strobila (× 5). *B*, Lateral branching showing terminal nub and reversed orientation of segments (× 10). *C*, Lateral branching with area of dedifferentiation (× 10). *D*, Detail of zone of granulation or dedifferentiation (× 110). *E*, Multiple branching, with division of segments of the most posterior branch (× 5)

Three groups of five grain beetles (*Tribolium confusum*), previously starved for seven days, were fed on abnormal gravid branches. No cysticercoids developed, owing either to reduced viability or insufficient numbers of eggs. However, normal cysticercoids developed when whole branched worms were exposed on filter paper to previously starved beetles. These cysticercoids produced only normal worms when fed to *DBA*/1 (Jackson strain), Heston/*A*, or Swiss Webster mice. Infections using grain beetle intermediate hosts were continued over four generations with the same result.

This phenomenon may represent a mutation activated by a specific host strain or an extraordinarily specific direct host-influence on an otherwise normal parasite. In either case, the high degree of dependence on the host for the expression of this abnormality emphasizes a remarkable specificity and an extreme degree of response of a parasite to its host.

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Calories/gm. in Species of Animals

ANIMALS of seventeen species, representing six phyla, were burned in a miniature bomb calorimeter[1]. The data are presented as calories/ash-free gm. in Table 1 and as a histogram, Fig. 1.

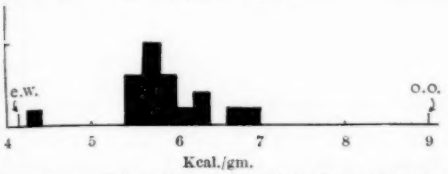

Fig. 1. Frequency distribution of the data in Table 1. The caloric values of egg white (e.w.) and olive oil (o.o.) have also been indicated.

Given a haphazard collection of species, any one of three mutually exclusive frequency distribution patterns might have been expected. These are listed below with the rationale of each :

(1) A normal, symmetrical distribution. This might be anticipated from the known biochemical similarities between animals. The variance would be related to slight biochemical differences and to errors of measurement.

(2) A skewed distribution with the modal frequency at or near the lower range limit. This might be anticipated, since there has always been selection for maximum number of reproducing progeny, but only sporadic selection for high-energy content/gm.

(3) Fairly great differences between major taxonomic groups with essentially normal distributions within each group. Many other properties of animals are common to a taxonomic category and differ between categories. Cal./gm. might be one such property.

The biological implications of the three possible distributions differ. The first distribution would imply that there is an optimal value of cal./gm., any deviation in either direction being selected against.

The second distribution would imply that there is some unique combination of components, characterized by a particular range of cal./gm., which just permits animals to maintain themselves. These components are essentially the same for all animals, and any combination with a lower cal./gm. value is non-viable. Higher values would be expected in animals that have temporarily accumulated fat, either because of unusually high food availability or as preparation for some period of fasting or stress.

Table 1. CAL./ASH-FREE GM. OF SEVENTEEN SPECIES OF ANIMAL

Common name	Species	Mean cal./ash-free gm.	± 95 per cent confidence limit of the mean (corrected for small sample size)
Ciliate	*Tetrahymena pyriformis*	5,938	± 207
Hydra	*Hydra littoralis*	6,034	± 146
Green hydra	*Chlorohydra viridissima*	5,729	± 247
Flatworm	*Dugesia tigrina*	6,286	± 338
Terrestrial flatworm	*Bipalium kewense*	5,684	± 124
Aquatic snail	*Succinea ovalis* (without shell)	5,415	± 6
Brachiopod	*Glottidia pyramidata*	4,397	± 2,140
Brine shrimp	*Artemia* sp. (nauplii)	6,737	± 863
Cladocera	*Leptodora kindtii*	5,605	± 584
Copepod	*Trigriopus californicus*	5,515	± 277
Copepod	*Calanus helgolandicus*	5,400	± 197
Caddis fly	*Pycnopsyche lepido*	5,687	± 530
Caddis fly	*Pycnopsyche guttifer*	5,706	one determination
Spit bug	*Philenus leucophthalmus*	6,962	± 510
Mite	*Tyroglyphus lintneri*	5,808	± 446
Beetle	*Tenebrio molitor*	6,314	± 516
Guppie	*Lebistes reticulatus*	5,823	one determination

The third distribution would imply that cal./gm. is a secondary property in the evolution of animals and that the cal./gm. in any animal is a by-product of other selective requirements. This would be expected if, and only if, energy has generally not been limiting during the course of evolution.

So far as we have gone, the results suggest the second alternative distribution listed. Twelve of the seventeen determinations give results of 5,400–6,100 cal./gm. The one low value is from a moribund brachiopod (*Glottidia*). Two of the four high values were from animals that were about to go through a fast, the *Artemia* nauplii, since they were unable as yet to feed, and the *Tenebrio* larva, since it was about to pupate. The other two were from very well-fed situations, the *Dugesia* being from a laboratory stock culture fed on *Artemia* nauplii and the *Philenus* being taken from the field in late summer. The *Philenus* specimens included egg-laden females, but, unfortunately, they were not sexed on collecting. It seems likely that many herbivorous insects normally have superabundant food available[2].

Cal./gm. may provide an index of nutritional condition of animals in the field which would be considerably cheaper and as reliable as the elaborate sampling programmes now required.

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Manganese and Magnesium in the Grey Speck Syndrome of Oats

IT is generally recognized that the grey speck syndrome in oats is caused basically by manganese deficiency[1]. Opinions differ, however, on whether grey speck symptoms are due directly to manganese deficiency[1–3], or whether manganese deficiency functions indirectly by rendering the roots of the plant more susceptible to infection by rhizosphere organisms the toxic products of which give rise to the visual symptoms[4].

In consequence, the possibility of selecting breeding material resistant to grey speck is complicated, despite the variation in resistance reported by several investigators[5–7]. The present work is concerned with the investigation of possible differences in the critical levels of manganese in tissues of varieties of oats susceptible and resistant respectively to grey speck. For this purpose, the hexaploid oat variety Star (susceptible) and the diploid variety *S*.171 (resistant)[8] were used and grown in sand culture with a modified Shive solution and six levels of manganese, 0·0, 0·1, 0·2, 0·5, 2·0 and 5·0 p.p.m. Washed sand, de-ionized water and 'Analar' chemicals were used, but no elaborate precautions were taken to eliminate all manganese from the zero treatments.

Table 1. Manganese and Magnesium Content of Oats, Susceptible and Resistant to Grey Speck Disease

Manganese treatment	Variety	Percentage Manganese Roots	Manganese Leaves	Magnesium Roots	Magnesium Leaves
0·0 p.p.m.	Star	0·00050	0·00050	0·45	0·55
	*S.*171	0·00256	trace	0·29	0·35
0·1 ,,	Star	0·00098	0·00175	0·44	0·50
	*S.*171	0·00300	0·00044	0·30	0·57
0·2 ,,	Star	0·00240	0·00200	0·46	0·45
	*S.*171	0·00350	0·00048	0·06	0·26
0·5 ,,	Star	0·00250	0·00800	*	0·27
	*S.*171	0·00500	0·00440	0·054	0·22
2·0 ,,	Star	0·00253	0·02000	0·25	0·35
	*S.*171	0·01250	0·01920	0·048	0·22
5·0 ,,	Star	0·00150	0·03900	0·23	0·48
	*S.*171	0·01350	0·02800	0·068	0·42

* No data.

The results (Table 1) show that for mature plants the manganese content of the leaves of Star is consistently higher than those of *S.*171 at the lower manganese-levels, but with manganese treatments of 2·0 and 5·0 p.p.m. there is no significant difference. The reverse holds for the manganese content of the roots, as in every case *S.*171 has the higher manganese content, and the difference between Star and *S.*171 is greatest at the highest manganese concentrations. Despite the low levels of manganese obtained in the leaves of both varieties at the 0·0 and 0·1 p.p.m.-levels, no typical grey speck symptoms were observed even in the susceptible variety Star. In contrast, other workers have reported grey speck symptoms associated with leaf concentrations of manganese in excess of those reported[3,9]. Our results are more comparable to those of Lundegårdh, who reported concentrations as low as 1 p.p.m. without grey speck symptoms[10].

In regard to magnesium, the content of the roots shows major varietal differences and an interaction between magnesium and manganese. The variety *S.*171 contains less magnesium in the roots at all levels of manganese than Star, but both varieties show a decrease in magnesium content when the manganese supply changes from limiting to adequate. The difference in the magnesium content of the leaves is not so marked, however, the decrease being most significant in *S.*171.

The relationship to grey speck resistance of high-leaf, low-root manganese content in the susceptible variety Star, and the low-leaf, high-root manganese concentration of the resistant variety *S.*171, is not clear. The analyses are for fully mature plants and may not represent the situation in actively growing material. It is possible that the results represent the effect rather than the cause of varying susceptibility to the grey speck syndrome. It has been established that redistribution of mineral constituents from the shoot occurs during the maturation of oats[11]. Thus, if at maturity manganese passes from shoot to root, then the higher manganese content of the roots and the lower content of the leaves in *S.*171 may reflect the greater mobility of manganese in this variety. The comparative lack of mobility of manganese in Star is then indicated by the high-leaf, low-root manganese concentrations, due to retention of manganese in the leaves without redistribution to the roots. Taking this view, susceptibility to the grey speck syndrome is primarily due to lack of mobility of manganese within the plant.

An alternative is that the mature plant analyses represent the situation in the growing plant, and in this case the grey speck resistance of *S.*171 may be due to the comparatively high concentration of manganese in the roots inhibiting the invasion and multiplication of ammonium-forming rhizosphere organisms, either directly or indirectly through the medium of a root exudate. Resistance to grey speck has been attributed by Gerretsen[6] to the root system having greater resistance to saprophytic organisms. However, more effective mobility of manganese seems more likely to be the basis of superior grey speck resistance, in that the syndrome appears to be primarily a disorder of photosynthesis, with root deterioration as a secondary factor. This is borne out by the rapidity with which deficiency is overcome when manganese-deficient cereals are sprayed with manganese sulphate.

Further work is in progress to determine the relationship of manganese mobility to resistance to grey speck, and the possible interaction of manganese and magnesium.

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Dependence on Light of Geotropism in Plant Roots

Plants grown in non-porous containers frequently show a lower concentration of roots on the periphery of the block of soil than similar plants grown in porous containers[1], and there is sometimes a tendency for the direction of growth of these peripheral roots to be more nearly vertical in non-porous than in porous containers[2,3]. Gradients of water, nutrient or gas concentration, or of soil temperature, have been suggested as causes of these phenomena, but there is also the possibility that light has an effect, since many non-porous containers made of glass or plastics are transparent, whereas porous 'clay' pots are opaque.

Seeds of China asters (*Callistephus chinensis* var. Ostrich Plume), stocks (*Matthiola incana* var. *annua*), *Calendula officinalis* var. Radio, tomatoes (*Lycopersicon esculentum* var. Amateur) and cucumbers (*Cucumis sativus* var. Market Cross) were sown on July 6, 1960, and transferred to John Innes No. 1 potting compost in the experimental pots on July 15. The pots were of colourless polyethylene, which is highly transparent to all wave-lengths of visible and infra-red radiation, and half of them were made opaque by coating them with black paint, on the outer surface, and on the inner surface down to the level of the soil. Opaque and transparent pots were arranged alternately on a bench in a glasshouse with natural illumination. Three plants of each species and from each kind of pot were examined on July 29 and at intervals of 3–6 days thereafter. An examination consisted in removing the plant from its pot and applying a 'cross of Lorraine' (Fig. 1) marked

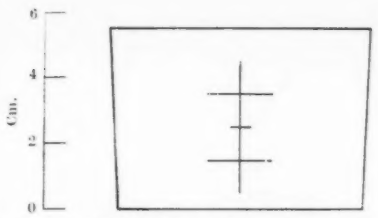

Fig. 1. Side view of a block of soil taken from a pot, with 'cross of Lorraine' superimposed for assessing roots

on a piece of transparent polyvinyl chloride, to four positions taken at random around the nearly vertical wall of the block of soil. In each position, the number of roots intersecting each of the two 2-cm. axes of the upper half of the cross and each of the two 2-cm. axes of the lower half were counted.

For all except tomatoes the increase, from one examination to the next, in the number of intersections counted was greater in the opaque than in the transparent pots. Thus, in order to compare the distributions of roots on the surface of the blocks of soil from the two kinds of pots when the total quantity of root present was much the same in both cases (Fig. 2), it was necessary to relate to the results from the transparent pots, obtained on August 15, corresponding results from opaque pots obtained respectively 16, 14, 6, 3 and 0 days earlier for stocks, calendulas, asters, cucumbers and tomatoes. One of the reasons for a low concentration of roots on the periphery of a block of soil from a transparent non-porous pot therefore appears to be that, for some species at least, light either inhibits the growth of the roots or causes them to turn back into the soil by negative phototropism.

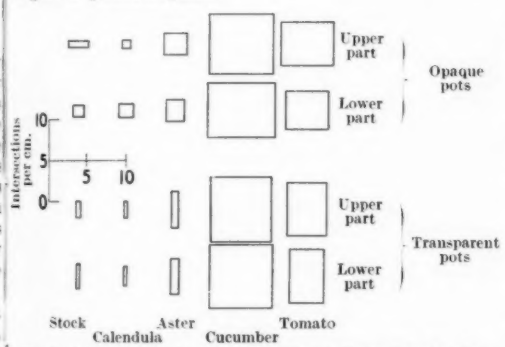

Fig. 2. Distribution of roots on the walls of blocks of soil taken from opaque pots and from transparent pots. The width of a rectangle represents the number of roots intersecting a vertical line and the height represents the number intersecting a horizontal line

Exposure to light influenced not only the concentration of roots but also their direction of growth, which was more nearly vertical in the transparent pots than in the opaque (Fig. 2). The difference was significant ($P<0{\cdot}001$) for all species, except cucumbers. The question arises as to whether this result is accurate or whether disturbing the roots, in order to examine them, had any effect upon the subsequent direction of growth. On August 22, plants of asters, stocks and calendulas which had been examined regularly (five times altogether) were compared with an equal number of plants which had been grown under similar conditions but which had not been disturbed. In opaque pots there was a greater tendency towards vertical growth in the disturbed roots than in the undisturbed of all three species, but the difference was only significant ($P<0{\cdot}01$) for asters (Table 1). For this species there was a similar and significant ($P<0{\cdot}01$) effect of disturbance in transparent pots, so that the result cannot be attributed only to the brief exposure to light at the time of examination, but also to the mechanical effect of taking the block of soil in and out of the pot. The roots in the upper part of the pot were those mainly affected.

Table 1. Effect on the Root System of *Callistephus chinensis* of Removing the Block of Soil from the Pot in Order to Examine It

Type of pot	Position of roots	Intersections of horizontal axis / Intersections of vertical axis: Disturbed	Undisturbed
Opaque	Upper part	0·514	0·423
Opaque	Lower part	0·524	0·524
Transparent	Upper part	0·758	0·650
Transparent	Lower part	0·787	0·772

The fact that transparency of the pot wall significantly ($P<0{\cdot}001$) affected the direction of growth of undisturbed roots (Table 1) establishes that the results shown in Fig. 2 cannot be explained by a difference between transparent and opaque pots in the number of times the roots were examined. For tomatoes there was in any event no such difference. The question therefore arises as to whether the downward growth of the roots in transparent pots was a negative phototropism or a light-induced geotropism. The rims of the pots were separated by 8·5 cm. on the glasshouse bench and the polyethylene of the pot walls was somewhat diffusing. The light falling on the roots was therefore coming from many directions, but always with a component into the soil, so that the main effect of phototropism would be a reduction in concentration of roots on the surface and it would only account for vertical growth where re-entry of the root into the soil was prevented. Such a reduction was observed for four species, but not in the case of tomatoes. Since there is no reason to suppose that the roots could not re-enter the soil, one is forced to the conclusion that the vertical growth was a response to gravity.

To confirm this, three plants each of stocks, asters and tomatoes were grown in transparent pots, on a piece of wire netting supported 20 cm. above a reflecting surface of aluminium foil. The pots were enclosed on all sides by a wooden box, and their tops were covered by a piece of foil, blackened on its lower surface and perforated with small holes through which the stems of the plants passed. The arrangement stood in a glasshouse in natural light, which could only reach the roots from below. Root-tips could be seen through the transparent walls of the pots, and their positions were marked without disturbing them. They grew downwards, although this was now definitely towards the light.

Main roots are commonly supposed to exhibit positive geotropism, and lateral roots are sometimes plagiogeotropic although the evidence for this is conflicting[4]. In the present experiment, in the opaque pots there was in no case any evidence for a preponderance of vertical roots; indeed there was even some evidence for a preponderance of horizontal growth (Table 1 and Fig. 1), especially in the upper part of the pot. In the transparent pots, on the other hand, all the species except cucumber showed a significant

tendency towards vertical root growth. Cucumbers commonly produce roots on the surface of the beds in which they are grown commercially.

It now appears that exposure to light is a prerequisite for geotropism in some plant roots. This could be because light is necessary for the formation in the roots of a growth inhibitor, which afterwards becomes asymmetrically disposed under the influence of gravity. The formation of such an inhibitor could also account for the reduction in concentration of roots on the surface of blocks of soil from transparent pots, whereas negative phototropism only does so with difficulty, since roots of stocks, which showed the greatest reduction in concentration, grew towards the light when illuminated from below. The failure of previous investigators to observe a light-dependence of geotropism may be accounted for by their use of very young seedlings, as there may then be sufficient of the necessary growth substance present in the radicle for a geotropic response to take place in the dark.

Thanks are due to L. G. Morris and R. W. Soffe for valuable advice and assistance.

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Mutation for Larger Fruit in the Raspberry

Raspberry breeders have long been concerned with the problem of breeding for larger fruit. Small fruits are a feature of wild raspberries from which present-day varieties are presumed to have been evolved by many generations of selection for improved size of fruit. It is therefore not surprising that size in raspberries is complex in its inheritance, and that breeders find difficulty in combining large fruit with the many other desirable characters required in modern varieties. The discovery of a mutant raspberry with larger fruits might be of importance as a means both of further improvement in this character and, if its inheritance were simple, of simplifying the breeder's task.

Such a mutant has been found in a commercial plantation of the variety Malling Jewel. It is characterized by an overall increase in the length and thickness of the fruiting laterals and the pedicels, enlarged floral parts, and an enlarged receptacle which carries fruit with more drupelets than the parent variety. The drupelets are also larger individually, and contain larger seeds. The laterals appear to develop more slowly, causing the fruit to ripen about 2 wk. later than the normal, but no differences have been found to distinguish the vegetative (first year) growth of the mutant from that of the parent stock.

Examination of pollen mother-cells from the mutant showed the normal diploid complement of fourteen chromosomes. Open-pollinated fruits from a plant propagated from the mutant and grown in isolation were used to raise a progeny of 114 seedlings: these all made normal vegetative growth but segregation for mutant types of fruiting lateral was evident in the second year. In addition to forms similar to the parental mutant, more extreme forms occurred which ripened later and had even larger laterals with malformed and frequently with sterile flowers. Classification of the seedlings gave normal, mutant and extreme mutant forms in the ratio of 40 : 56 : 18. This does not depart significantly from the expectation if the mutation were caused by a change in a major gene from the homozygous to the heterozygous state, and if the latter were intermediate in expression.

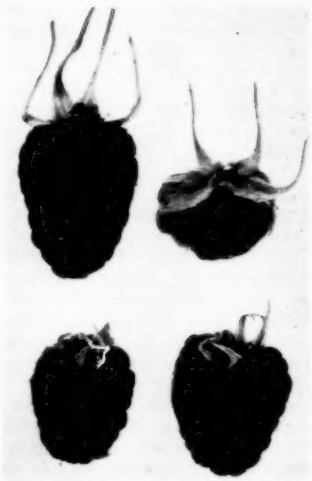

Fig. 1. Fruits of mutant Malling Jewel top left, mutant Malling Promise (top right), normal Malling Jewel (bottom left) and normal Malling Promise (bottom right)

It seems that the change causes an error in the development of the axillary meristems, resulting in enlargement of all the tissues derived from them. The manifold effects of the gene are probably the result of what Grüneberg[1] has termed 'spurious' pleiotropism. Certain of these effects, such as the increase in seed size, are undesirable, but the growth of fruiting laterals in raspberries is governed by many minor gene complexes which give considerable independent variability to each component part. It is probable that the faults could be corrected by selection for these minor genes, for example by selecting for small seeds after crossing the mutant with a small-seeded variety such as Baumforth's Seedling *B*.

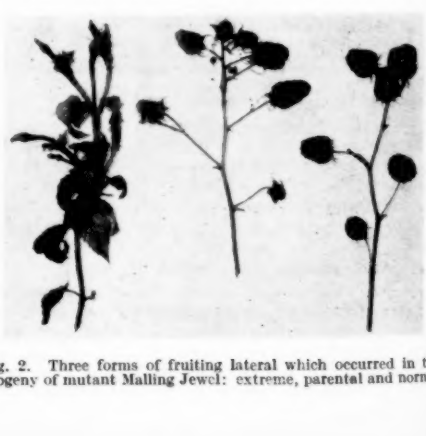

Fig. 2. Three forms of fruiting lateral which occurred in the progeny of mutant Malling Jewel: extreme, parental and normal

A second mutant raspberry has been found in a plantation of Malling Promise, but this mutant more closely resembles the extreme and presumably homozygous forms which segregated from the Malling Jewel mutant. It is conceivable that the same mutant gene may be more dominant in some genic backgrounds and may produce these sub-fertile forms when it is heterozygous; alternatively the Malling Promise form may be a higher allele. An investigation is being made of the interactions of the mutant gene with different minor gene complexes to clarify these questions.

I am indebted to Mr. J. Morrison, of Forfar, Angus, for directing my attention to these mutants in 1958 and 1959, and to Miss N. N. Tun for counting the chromosomes.

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Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of New Zealand Butterfat

MARKED and regularly recurring seasonal fluctuations in the characteristics of New Zealand butterfat have been reported by Cox and McDowall[1]. The iodine value, for example, rises to a maximum of about 41 in mid-winter, falls gradually until the beginning of October and then rapidly during October and November to a mid-summer minimum of about 34, and rises again during the autumn and early winter to the mid-winter maximum. The carotene and vitamin *A* contents of the butterfat also have been shown by McDowell and McDowall[2] to change according to a regular seasonal pattern, with maximum values in the early spring and minimum values in mid-summer. These latter changes have been found by McGillivray[3] to be a reflexion of changes in the carotene and vitamin *A* contents of the blood plasma.

Seasonal changes in the properties of butterfat resulting from alternations between stall and pasture feeding of the cows, or from changes in the type of feeds given to stall-fed cows, have been reported by numerous investigators overseas, but it was surprising to find changes of this magnitude in the butterfat and blood plasma of cows grazing throughout the year on open pasture according to the general New Zealand practice. The causes of these changes have not hitherto been established.

In New Zealand almost all cows supplying the dairy manufacturing industry are calved down in the early spring. There are therefore consistent changes in the stage of lactation of the cows with progress of the dairying season, both for successive dairying seasons and for the different dairying districts. It is then a facile inference that the changes in butterfat properties are associated with change in the stage of lactation of the cows. In an investigation at this Institute with monozygous twin cows separated by several months in calving date, however, the butterfat from the later calving cow of each pair of twins was similar in properties to that from the earlier calving cow after the normal immediate post-parturition changes had been completed.

The indications were, therefore, that the changes in butterfat properties resulted from changes in the nature of the feed consumed by the cows, that is, changes in the character of the pastures. In this connexion it seemed significant that the sudden change in properties of butterfat in October–November is coincident with the rapid expansion in growth of clover in the clover-ryegrass associations, typical of most dairy pastures in New Zealand.

A change from ryegrass-dominant to clover-dominant pasture has been found by Worker and McGillivray[4] to cause some decrease in vitamin content of the butterfat, but the effect was relatively small and somewhat irregular. Further trials in which ryegrass and white clover were fed separately to twin cows have indicated that the effect of the sudden increase in the proportion of clover in the pasture consumed by cows is not sufficient to account for the observed changes in the characteristics of the butterfat.

At the same time as the proportion of clover in the mixed pasture shows a rapid increase, there is, during October–November, a transition in character of the ryegrass from the early spring lush soft foliage to the somewhat harder and more fibrous foliage which develops as the grass matures. Pure stands of *H*1 short rotation ryegrass were therefore prepared, for an investigation of the effect of maturity of ryegrass in the pasture on the butterfat properties. Portions of the area were kept closely grazed, so that the leaves were short (3–4 in.) and succulent, and the grass on the remainder of the area was allowed to grow to a height of approximately 12 in., at which stage the stalks were firming and becoming somewhat fibrous. Cows from a number of monozygous twin pairs were grazed on the two areas during September–December. Throughout this period it was found that the butterfat from the cows on the young leaves had the properties of normal early spring New Zealand butterfat, whereas that from the cows grazed on the longer-stemmed ryegrass had the properties and the carotene and vitamin *A* contents of normal October–November New Zealand butterfat. There were similar differences in the carotenoid contents of the bloods of the two groups of cows. The differences were maintained over the whole period of feeding of the two types of pasture. The average results given in Table 1 cover the period during which more detailed investigations of the fatty acid composition of both grass lipid and butterfat were made. Although the fats from both types of pasture were highly

Table 1. AVERAGE ANALYTICAL RESULTS FOR GRASS, GRASS LIPIDS, BLOOD AND BUTTERFAT FOR THE TWO TREATMENTS DURING SEPTEMBER 25–OCTOBER 6

Product analysed	Substance estimated	Average results		Difference (*I*)–(*M*)
		Immature grass (*I*)	Mature grass (*M*)	
Grass	Carotene (μgm./gm. dry matter)	545	400	145
	Lipid content (mgm./gm. dry matter)	86	46	40
Grass lipid	Linolenic acid (moles per cent)	62·0	53·0	9·0
Blood	Carotene (μgm./ml.)	21	15	6
Butterfat	Carotene (μgm./gm.)	11·5	10·3	1·2
	Vitamin *A* (μgm./gm.)	10·6	8·1	2·5
	Iodine value	43·2	35·6	7·6
	Oleic acid (moles per cent)	34·3	30·5	3·8
	Linoleic acid (moles per cent)	3·3	1·8	1·5
	Linolenic acid (moles per cent)	1·5	1·0	0·5

unsaturated, those from the immature pastures contained a higher proportion of unsaturated acids than those from the mature pastures. There was also a higher proportion of fat in the immature pasture. The differences in properties of the butterfats from cows grazing on the two types of pasture were thus associated with differences in both the nature and the amount of lipids present. It would seem, therefore, that the composition of butterfat from pasture-fed cows is markedly influenced by the type of pasture consumed, and that the seasonal changes in the carotene content of the blood and in the composition and properties of the butterfat from cows grazing typical New Zealand pastures result from seasonal changes in the state of maturity of the ryegrass in the pasture.

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Intra-ovarian Pollination in *Eschscholzia californica* Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet

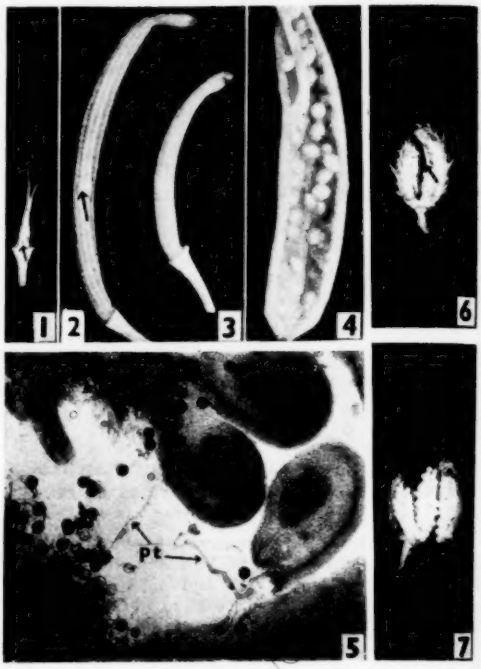
In an earlier communication one of us[1] described the results of intra-ovarian pollination in *Papaver rhoeas*. The present work deals with some observations of a similar nature on *Eschscholzia californica*, *Argemone mexicana* and *A. ochroleuca*.

The pollen suspensions were prepared in 0·5 c.c. of a boric acid solution—200 mgm./l. for *Eschscholzia* and 100 mgm./l. for *Argemone*. Each drop of the suspension contained 200–300 pollen grains.

Eschscholzia. The ovaries injected with pollen suspension grew normally (Figs. 1–3) although the size of the fruits was smaller than that of fruits from naturally pollinated ovaries.

The pollen grains germinated in the ovarian cavity (Fig. 5) and many pollen tubes entered the ovules. The growth of the fertilized ovules was slow in the beginning but the ultimate size (Fig. 4) attained by them was equal to that of ovules from naturally pollinated ovaries. The maturation of the ovaries and dehiscence of the capsules resulting from intra-ovarian pollination took almost the same time as in Nature (25–30 days). The seeds were viable and the embryos as large or larger than those which developed naturally. The seeds germinated and gave rise to normally flowering and fruiting plants.

Argemone. When a pollen suspension in 100 mgm./l. boric acid was injected the germination of the pollen grains inside the ovarian cavity was very low and scarcely more than 1 or 2 seeds were produced per fruit, or sometimes none at all. If a suspension of the pollen grains in double distilled water was used, seed setting was slightly improved (2–4 per fruit). However, when the pollen grains were introduced as such into the ovary through a vertical slit, they germinated profusely (50 per cent) and the seed set was very high. In *A. ochroleuca* about 60 seeds (Figs. 6, 7) and in *A. mexicana* as many as 140 seeds were produced per fruit; in Nature the number of seeds varies between 150 and 200 in the two species.



Figs. 1–5. *Eschscholzia californica*, ovaries injected with a suspension of pollen in 200 mgm./l. boric acid. Fig. 1. Ovary at the time of anthesis and injection of pollen grains; the arrow in this and in Fig. 2 indicates the place of injection of the pollen suspension (× 2). Figs. 2 and 3. 17 and 25 days after injection (× 2). Fig. 4. Same as Fig. 3, cut vertically to show the developing ovules (× 2·25). Fig. 5. Transverse section of ovary 72 hr. after injection; note the pollen tubes (*pt*) from the germinating pollen grains in the ovarian cavity (× 47). Fig. 6. *Argemone ochroleuca*, ovary 12 days after insertion of pollen through a vertical slit shown by an arrow (× 2). Fig. 7. Same as Fig. 6, split open to show developing ovules (× 2)

In *A. mexicana* the growth of treated ovaries was slow and the maximum size attained by them was only two-thirds of the normal. In *A. ochroleuca* the treated ovaries grew to less than one-half of their natural size. Nevertheless, in both the species the growth of the ovules and the differentiation of the embryo were almost similar in the treated as well as in the naturally pollinated ovaries. The capsules ripened and dehisced in the same period (27–30 days after anthesis) in both sets and the seeds obtained by intra-ovarian pollinations were fully viable.

A. mexicana is a diploid ($n = 14$) and *A. ochroleuca* a tetraploid ($n = 28$)[2,3]. Natural hybrids between the two are rare and usually sterile[2]. This incompatibility could possibly be overcome by intra-ovarian pollination and work in this direction is in progress. The technique of injecting pollen grains into the ovary may be of help in overcoming some types of incompatibilities.

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CYTOLOGY

Periodic Acid–Schiff Positive Giant Cells in the Mouse Thymus Cortex

HAMMAR[1] first described cells, laden with fat granules, in the thymus cortex of ageing humans. More recently, a detailed description of the histochemical reactions of these cells in the mouse thymus has been given by Loewenthal and Smith[2], who pointed out that these cells gave a strong red colour when stained with the periodic acid–Schiff reagent (PAS).

During a survey of the morphology of the thymus in high- and low-leukæmia strain mice, these cells were observed in large numbers in the cortices of ageing *AKR* and *C3H* mice. Their nuclei resembled thymic reticulum cell nuclei rather than those of the lymphocyte series. The cytoplasm of these cells was voluminous, and packed with granular material with the following staining reactions: positive to PAS (diastase resistant), pyronine and scharlach *R*; negative to toluidine blue (stained pale green), eosin and Feulgen. The cells appeared capable of phagocytosis, since they frequently contained pyknotic nuclear material, presumably lymphocytic in origin. These cells first appeared in the thymus in mice aged between two and four months, and their number increased with advancing age, although varying considerably in individual thymuses. These cells were found in increased numbers in thymuses of mice in the second half of pregnancy, if aged older than four months. The development of these PAS cells appeared to be related to changes in age of cellular elements within the thymus itself. One-week-old thymuses were grafted subcutaneously into six-month-old *C3H* mice and examined histologically one month after grafting. Although all host thymuses showed numerous PAS cells, none of the grafted thymuses showed such cells. Similar negative results were obtained with week-old thymuses grafted to pregnant six-month-old *AKR* hosts. These results appear to exclude the possibility that circulating humoral factors in ageing mice are primarily responsible for the development of these cells.

The location of the PAS cells was usually subcapsular, but frequently they were scattered uniformly throughout the cortex. The cells were not uniform in size and varied from 7μ to 26μ in diameter. The mean size of the cells increased progressively with advancing age. The PAS cells were surrounded by tightly packed lymphocytes. Frequently lymphocytes were seen which had invaginated the cytoplasm of the PAS cells and were lying in deep crypts, projecting into the cells.

In 7μ sections, one in three PAS cells was observed to have one or more mitotic lymphoid cells in contact with some part of the cytoplasm of the cell. A quantitative analysis was made of the frequency of mitotic lymphoid cells in the area surrounding PAS cells, relative to areas distant from these cells. In each thymus, twelve fields, 72μ × 72μ, were selected at random and drawn on graph paper and the total PAS area determined (area of PAS cells, plus area 5μ in width around each cell). Cortical tissue outside this area was designated 'non-PAS' area. The number of mitoses in both areas was determined and the stage of each mitosis noted. The results (Table 1) indicated that, in *AKR* thymuses, mitoses per unit area were three times more frequent in the PAS areas than in the adjacent 'non-PAS' areas. The results in thymuses of the low-leukæmia strain *C3H* indicated much less difference in the incidence of mitoses between the two areas. These results require extension to confirm the reality of this difference in strain.

Table 1

Strain	Age (months)	Sex	No. of thymuses	Mean percentage PAS area	Mean mitoses in PAS area, per unit area	Mean mitoses in 'non-PAS' area, per unit area	Ratio PAS mitoses 'non-PAS' mitoses*
AKR	6–10	M	13	27	95	32	3·1 ± 0·8
AKR	6–8	F	10	21	73	26	3·0 ± 0·8
C3H	8–10	M	12	24	59	42	1·4 ± 0·4

* ± *S.D.*

The analysis of the percentage of cells in various stages of mitosis in the two areas revealed, in *AKR* thymuses, a predominance of prophase mitoses in the PAS areas (11 ± 4 per cent versus 6 ± 3 per cent in the 'non-PAS' areas). The percentages of the other stages of mitosis were similar in both areas.

The significance of the association between lymphocyte mitotic activity and PAS cells in the thymus cortex is not known. The PAS cells may either stimulate mitosis in neighbouring lymphoid cells, or may attract cells about to enter mitosis. The predominance of prophase mitoses around PAS cells suggests that such stimulation or attraction may be related to the initial phases of mitosis, and that some of the mitotic cells may later move away from the PAS cells before mitosis is complete.

Since the PAS cells and associated mitoses occur only in post-involutional thymuses, they may represent some type of regulation of thymic lymphocyte mitotic activity, supervening on an earlier form of regulation present in the pre-involutional thymus.

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VIROLOGY

A Method of treating Asbestos Filter Pads for Virus Filtration

PRE-TREATMENT of Seitz-type filters with serum or with digest broth will reduce, to some extent, the often serious losses of virus caused by adsorption during filtration. Solutions of gelatin have been used recently instead of serum or broth with the same object in view. Although certain advantages are claimed for the use of gelatin, for example that most samples are antigenically inert, experience has shown that losses during filtration through asbestos filters treated with this protein substance are not uniformly negligible.

For some purposes it is desirable to avoid the addition of foreign protein to virus preparations, and so investigations were made to determine whether

non-protein substances of complex molecular structure would diminish the virus-adsorbing properties of asbestos filter pads.

Sodium alginate, the sodium salt of alginic acid $(C_6H_9O_6)_n$, a polymer of beta-D-mannuronic acid, which is generally considered to be a non-protein substance, was used for this purpose and its efficacy was compared with that of gelatin. It is as well to mention, however, that protein estimations by the Folin-Ciocalta method revealed that the particular preparation used in these experiments contained a trace of protein (0·16 per cent calculated for dry weight). This protein no doubt occurred as an impurity and originated from the seaweed *Laminaria cloustoni*, from which the alginate was prepared. The concentration of protein in the 0·04–0·05 per cent solutions finally recommended for treatment of filter pads would be only 0·0006–0·0008 per cent. The effect of the alginate solution is attributable, therefore, principally to the complex polysaccharide molecule of alginic acid, in which the mannuronic acid units are connected in long chains by 1,4-β-glycosidic linkages.

Asbestos filter pads were treated before use with 25·0 ml. of solutions containing concentrations of either sodium alginate or gelatin in the range 0·0025–0·06 per cent; excess solution was then washed from the pads by passing 50·0 ml. of *M*/25 phosphate buffer through them. Untreated control pads were washed with 75·0 ml. of buffer only, to adjust the *p*H of the pad.

Preparation of viruses. The virus suspensions used in the experiments were first passed through clarifying filters to remove gross particulate matter. The results obtained therefore refer to relatively crude preparations and not necessarily to highly purified virus.

For all experiments on the virus of vesicular stomatitis, an egg-passaged strain of Indiana type (Ind. *C.*) was used, manipulations being performed with appropriate precautions to avoid inactivation by light[1]. The virus suspensions in physiological saline were made from embryo extracts.

Strains of foot-and-mouth disease virus of types *O*, *C* and Asia 1 were propagated in serum-free pig kidney or tongue epithelium tissue culture; culture extracts were diluted in *M*/25 phosphate buffer before filtration.

Filters. Asbestos filter pads of 3·0 cm. diameter were used throughout the series of experiments; all were of *E.K.* equivalent grade (Ford *G.S.* or Seitzwerke *E.K.*). Solutions of gelatin were made by dissolving gelatin powder (British Drug Houses, Ltd., Poole, Dorset) in warm distilled water, and then diluting the solution to give the required concentration of gelatin. The sodium alginate used in all experiments was of pharmaceutical grade and conformed with the *British Pharmaceutical Codex* specification. The following analytical data were kindly supplied by the makers (Messrs. Alginate Industries, Ltd., Bedford Street, London, W.C.2):

Moisture content		20·4 per cent
Calcium content		0·27 per cent
Lead content	(less than)	10 p.p.m.
*p*H		6·3
Viscosity of 1 per cent solution*		62 centi-stokes

* Measured in a B.S. U-tube viscometer at 25° C. and corrected for moisture content of the powder.

The calculated mean molecular weight was approximately 88,000; somewhat greater than that of serum albumins (65,400–72,300 when calculated from sedimentation and diffusion constants[2]).

Stock solutions of sodium alginate in glass-distilled water were prepared at a concentration of 0·05 or 0·1 per cent w/v, and these solutions were diluted for use as required. Residual solutions were always discarded seven days after preparation.

Mice. Samples of vesicular stomatitis virus were titrated in 8-day old mice of the Pirbright '*P*' strain; 4–6-day old mice of the same strain were used for titrations of the foot-and-mouth disease virus.

Titrations were made in groups of 8 or, occasionally, 6 mice per dilution with either 0·6 or 0·5 log intervals between dilutions of each series. The dose was 0·03 ml. for both virus systems, but inoculations were made intracerebrally with vesicular stomatitis virus and intraperitoneally with foot-and-mouth disease virus.

Calculation of results. The 50 per cent fatality end-point for each series was estimated by the Spearman-Kärber method to permit determination of the amount of virus lost during filtration.

The results obtained with suspensions of vesicular stomatitis virus before and after filtration are given in Fig. 1. They show that when filters are merely washed with *M*/25 phosphate buffer, to prevent inactivation of virus by *p*H changes while passing through the filter, the losses of virus during filtration in some cases exceeded 5 log units.

Fig. 1. Filtration of vesicular stomatitis virus through treated asbestos filters. Ford *G.S.* filter, ●; Seitz *E.K.* filter, ○; sodium alginate solution, ——; gelatin solution, – – –

Pre-treatment of filters with increasing concentrations of either gelatin or sodium alginate progressively decreased the losses of virus up to a finite value for each substance; but thereafter further increments of gelatin or sodium alginate in the solution failed to reduce filtration losses below the critical values of 1·95 and 0·9 log units, respectively. Losses in gelatin-treated filters were always greater than losses in filters treated with the same concentration of sodium alginate (Fig. 1).

When foot-and-mouth disease virus was filtered through either treated or untreated filters, the adsorption losses were considerably less than those encountered during filtration of vesicular stomatitis virus. This difference is probably a function of virus size, the

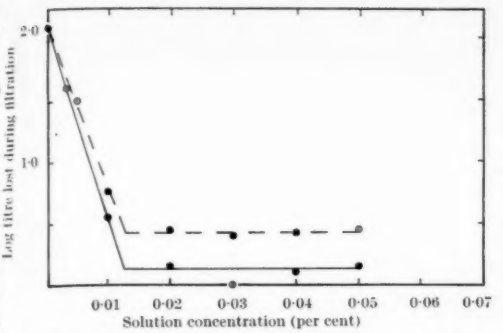

Fig. 2. Filtration of foot-and-mouth disease virus through treated asbestos filters. Mean values, ●; single values, ○; sodium alginate solution, ——; gelatin solution, – – –

infective component of the former virus being a roughly spherical particle 20–25 mμ in diameter, whereas that of the latter virus is a short rod-shaped body having an effective filtration diameter of 80–100 mμ. Nevertheless, more virus was lost in pads treated with gelatin solutions than in the corresponding pads of the sodium alginate series (Fig. 2). Irrespective of the concentrations used, it was impossible to reduce the filtration losses to less than about 0·15 log unit with sodium alginate or 0·43 log unit with gelatin.

These observations, together with the apparent inability of sodium alginate to act as an antigen, indicate that pre-treatment of asbestos filter pads with 0·04–0·05 per cent solutions of sodium alginate is a valuable means of minimizing adsorption losses during filtration of virus suspensions. The inferior results obtained with the protein, gelatin, may justify attribution of this effect to the sodium alginate *per se*, and not to the presence of infinitesimal amounts of protein impurity in the solutions used for treatment of the filters.

I am indebted to Dr. F. Brown for the protein estimations.

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SOIL SCIENCE

Factors influencing the Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates

Although many soil micro-organisms can decompose cellulose[1], each group of organisms does so under its own set of conditions. For example, *Cellvibrio* and *Cytophaga* develop around soil crumbs placed on filter paper which covers a mineral silica-gel medium, as shown by Winogradsky[2]. Other workers have reported that cellulose-decomposing fungi were the first organisms to attack 'Cellophane' buried in the soil[3].

It was sought to determine what causes one or the other species of the cellulose-decomposing flora to predominate and whether this is due to differences in technique or in the cellulose substrate used. For this purpose the composition of the cellulose-decomposing soil flora on filter paper and on 'Cellophane' were compared under the same conditions. Strips of filter paper (Whatman No. 1) and strips of 'Cellophane' (*PT* 300, British Cellophane Co.) were placed on mineral agar solidified in Petri dishes. Soil crumbs taken from the same soil sample were spread on strips of filter paper and 'Cellophane', respectively, and the Petri dishes were incubated for a few days at 30°.

Bacteria only (mostly *Cellvibrio*) developed on the plates covered with filter paper, while cellulose-decomposing fungi (mostly *Stachybotrys*) predominated on 'Cellophane' plates.

The failure of *Stachybotrys* to appear around soil crumbs on the filter paper could not be explained by its inability to attack the substrate, because a pure culture of *Stachybotrys* grew well on filter paper. To elucidate this question, the possibility of the bacterial growth interfering with the fungal growth was investigated. Soil crumbs were spread on filter paper placed on mineral agar plates containing 100 μgm./ml. chloramphenicol to inhibit bacterial growth, when only cellulose-decomposing fungi, especially *Stachybotrys*, developed.

In further tests a pure culture of *Stachybotrys* spores was mixed with a pure culture of *Cellvibrio* and the mixture inoculated on strips of filter paper on mineral agar. Under these conditions *Stachybotrys* failed to develop, or grew poorly, demonstrating the strong inhibitory effect of *Cellvibrio*.

Dobbs and Hinson[4] show that soil has a strong inhibitory effect on the germination of fungal spores; thus it was of interest to see whether *Cellvibrio* inhibited the germination of *Stachybotrys* spores. Direct microscopic observations of *Stachybotrys* spores in the presence of *Cellvibrio* showed that, although the spores germinated normally, growth was arrested after the development of the germination tubes. *Cellvibrio* inhibited the growth of the fungus only on media containing cellulose as the sole carbon source, and not when glucose or cellobiose was present.

No filtrable or diffusible substances responsible for the antagonism were detected. Direct contact between both organisms seems to be necessary to get the effect.

The predominance of fungi in the flora of soil crumbs spread on 'Cellophane' could easily be explained when it was shown that a pure culture of *Cellvibrio* either failed to grow, or grew only slightly, when 'Cellophane' was used as the substrate.

'Cellophane' has been much used recently in investigations of the cellulose-decomposing soil flora[3]. It is therefore important to point out that this substrate does not equally support the development of all the cellulose-decomposing micro-organisms, but seems to be more of an enrichment medium for the selection of cellulose-decomposing fungi.

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XUM

PSYCHOLOGY

Learning in Two Sense-modalities

In previous investigations[1,2] monkeys were taught to discriminate between shapes or rates of intermittence exclusively in one sensory modality (say visual) and were afterwards tested with the same shapes or rates exclusively in another modality (say tactile for shapes or auditory for rates). There was no evidence of cross-modal transfer of training. A variation of this procedure was also briefly described[1]. Four animals first learnt to discriminate between two test objects exclusively by touch (in the dark) and were afterwards tested for 30 trials with the same objects (in the light) under conditions where the objects could be discriminated either by touch or by vision. Only one animal performed significantly better than chance in the light. It was concluded that "even when monkeys are constrained to touch the very cues which served as the basis for successful somato-sensory discrimination of shape or size on the preceding day, there is still no clear indication of positive transfer on visual testing" (p. 62). In the present investigation the converse procedure was used: animals were first trained to discriminate between test objects by vision and/or touch (in the light) and were afterwards tested with the same objects for discrimination by touch alone (in the dark).

Three separate batches of respectively 8, 6 and 7 untrained rhesus monkeys (weight-range, 3·3–6·3 lb.) formed the three groups of subjects. All the subjects in a group were trained concurrently and all training was carried out under comparable conditions by the same investigator. However, group I animals were trained during March, group II during July and group III during November. (This consecutive training was necessary because all animals afterwards took part in different experiments.)

Standard apparatus (Wisconsin general testing apparatus) was used in conjunction with two food boxes placed 12 in. apart. The test objects were fastened to the lids of these boxes. Forty trials (non-correction) were given each day with the right/left positions of the positive test objects determined by the Gellerman schedule. During tactile training (in the dark) the animals' performance was observed by means of an infra-red telescope.

Animals of group I were first taught (see Table 1) to discriminate in the light between two relatively unsaturated hues, pink and cream. Animals of groups II and III were first trained on a quality discrimination in the light. In this test the positive (rewarded) object consisted of a piece of black foam rubber (2 × 0·75 × 0·3 in.) attached across the lid by a steel strip and two bolts. The negative object consisted of a black piece of wood of nearly the same dimensions as the rubber (but of different texture). The lids could only be opened by grasping and then pushing either the rubber or the wood. For their second test all 21 animals were trained to make this quality discrimination in the dark. However, animals of group III were concurrently given a further 10 trials on the visual form of this test according to a method described elsewhere[3]: visual trials in the light alternated with tactile trials in the dark (making 20 visual and 20 tactile trials each day), but were excluded from the learning scores relating to the tactile form of the test.

Table 1. Training Histories and Results on Quality Discrimination Test

Group I (n = 8)	Group II (n = 6)	Group III (n = 7)
Visual hue discrimination Mean = 275 trials	Quality discrimination (in the light) Mean = 192 trials	
Tactile quality discrimination test (in the dark)		
Animal: (1) 137 trials	93 trials	85 trials
(2) 162 ,,	116 ,,	55 ,,
(3) 133 ,,	98 ,,	243 ,,
(4) 157 ,,	97 ,,	43 ,,
(5) 60 ,,	84 ,,	318 ,,
(6) 108 ,,	120 ,,	46 ,,
(7) 124 ,,		62 ,,
(8) 161 ,,		

The figures refer to the number of trials required to reach the standard level of performance (10 or less errors in 100 consecutive trials).

Table 1 contains the results for the tactile test. There is considerable overlap between the scores of animals belonging to different groups. Animals of group I tended to have the highest scores and animals of group III, with two exceptions, tended to have the lowest scores. These two animals, although extremely hesitant and unresponsive on this their first tactile test, have not been excluded because on subsequent tactile testing their performance fell within the range relating to other animals. Statistical comparisons (Mann-Whitney 'U' test) indicate a significant difference between the scores of group I and group II animals (P = 0·02); but not between the scores of group I and group III animals (P = 0·14) or between those of group II and group III animals (P = 0·15).

These results indicate that in the monkey combined visual and tactile experience of two objects may lead to significantly improved performance (group II animals) when the same objects are afterwards offered for exclusively tactile discrimination. A comparable investigation has not been found in the literature. There are at least three interpretations of the present results. First, since cross-modal transfer from vision to touch could not be demonstrated in a previous investigation[1] it is unlikely that memory of the actual visual quality discrimination improved the performance of the group II animals during later tactile testing in the dark. Secondly, non-specific familiarity with the test objects, acquired during training in the light, may have accelerated subsequent tactile learning. Thirdly, it is possible that obligatory tactile contact with the objects during testing in the light led to some measure of genuine tactile discrimination learning. Irrespective of its origin, this improvement in tactile learning resulting from visual-tactile experience of the test objects in the light was not as pronounced, at least under the present experimental conditions, as might have been expected.

This work was supported by the Research Fund of the Institute of Neurology and by the Medical Research Council. Dr. E. A. Carmichael gave it his active encouragement and provided facilities, as did Dr. E. T. O. Slater. I am also much indebted to Dr. S. L. Chorover for help with the preparation of this communication.

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FORTHCOMING EVENTS

Tuesday, July 18

COUNCIL FOR THE PRESERVATION OF RURAL ENGLAND (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 2.30 p.m.—Annual General Meeting. Mr. Harold Abrahams, C.B.E.: "The Amenity Societies, the National Parks Commission and their Common Cause".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT, Grade "B" (with a good honours degree, together with some research, industrial or teaching experience, and preferably a knowledge of some branch of modern applied mathematics such as operation research, numerical analysis or electronic computers) IN MATHEMATICS—The Principal, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3 (July 19).

LECTURER (Grade II) or ASSISTANT LECTURER (Grade III) IN CLINICAL PATHOLOGY IN THE DEPARTMENT OF VETERINARY MEDICINE at the School of Veterinary Science, Langford—The Registrar and Secretary, The University, Bristol (July 19).

RESEARCH SOCIOLOGIST IN THE DEPARTMENT OF NUTRITION, for an investigation of social factors affecting nutrition—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 19).

LECTURER or ASSISTANT LECTURER (graduate with an interest in the philosophy of science) IN PHILOSOPHY—The Registrar, The University, Leicester (July 20).

ASSISTANT, Assistant Experimental Officer grade (preferably with a degree and some knowledge of languages), with the Agricultural Research Council's Weed Research Organization, near Oxford, for duties which include abstracting, indexing and responsibility for the Organization's Library—The Secretary, Agricultural Research Council, Weed Research Organization, Begbroke Hill, Sandy Lane, Kidlington, Oxford (July 22).

COMPUTER OFFICER (graduate) IN THE SUB-DEPARTMENT OF NUMERICAL ANALYSIS—The Registrar, The University, Liverpool (July 22).

GRADUATE (with a degree in biochemistry or related subjects), to take part in a chemical and biochemical investigation concerned with fruits and vegetables—The Director, Low Temperature Research Station, A.R.C., Downing Street, Cambridge (July 22).

LECTURER or JUNIOR LECTURER (with a postgraduate degree in biochemistry and some teaching experience) IN THE DEPARTMENT OF BIOCHEMISTRY—The Secretary, The London Hospital Medical College, Turner Street, London, E.1 (July 22).

LECTURERS (preferably with research and/or industrial experience) IN PHYSICAL CHEMISTRY—The Principal, Nottingham and District Technical College, Nottingham (July 22).

RESEARCH ASSISTANT (graduate in science, preferably physics, and with a knowledge of electronics), for investigations into the measurement of radioactivity in livestock—The Registrar, The University, Leeds 2 (July 22).

RESEARCH ASSISTANT IN THE DEPARTMENT OF GEOLOGY, to collaborate in field and laboratory studies of Irish Dalradian rocks—The Registrar, The University, Liverpool (July 22).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with a good honours degree, and preferably some postgraduate experience), to work on biochemical processes in seeds and seedlings—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (July 22).

TEMPORARY LECTURER IN PURE MATHEMATICS—The Registrar, University College, Singleton Park, Swansea (July 22).

RESEARCH STUDENT IN X-RAY CRYSTALLOGRAPHY, to work for a higher degree under the supervision of Dr. S. C. Nyburg on crystal structures and absorption spectra of transition metal complexes—The Registrar, University College of North Staffordshire, Keele, Staffs (July 28).

SCIENTIFIC OFFICER, SENIOR SCIENTIFIC OFFICER or PRINCIPAL SCIENTIFIC OFFICER (suitably qualified scientist, preferably with experience of blood grouping or serology), to extend the work of the Organization on antigens and biochemical variants in cattle blood—The Secretary, Animal Breeding Research Organization, Glenbourne, South Oswald Road, Edinburgh 9 (July 29).

LECTURER (medical or honours science graduate, with experience in general microbiology or microbial chemistry, and in research methods) IN BACTERIOLOGY—The Secretary, The University, Edinburgh (July 31).

LECTURER or SENIOR LECTURER (preferably with a special interest and experience in dairy husbandry and postgraduate experience in animal physiology) IN THE DEPARTMENT OF AGRICULTURE—The Registrar, The University, Leeds 2 (July 31).

PLANT PHYSIOLOGIST, Scientific Officer grade (with an honours degree and preferably postgraduate experience), to work on light intensity and leaf canopy development in orchards in relation to tree form and its modification for high yield—The Secretary, East Malling Research Station, Maidstone, Kent (August 1).

POSTDOCTORAL FELLOW IN THE DEPARTMENT OF CHEMISTRY, to work on mould metabolites—Prof. R. A. Raphael, Department of Chemistry, The University, Glasgow, W.2 (August 1).

SENIOR TECHNICIAN IN THE DEPARTMENT OF AGRICULTURE, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 4).

LECTURER or ASSISTANT LECTURER IN ZOOLOGY (ENTOMOLOGY) at the University College of The West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 7).

TECHNICIAN (qualified and experienced draughtsman, preferably with photographic experience) IN THE DEPARTMENT OF GEOGRAPHY, University College, Ibadan, Nigeria, for work involving the preparation of maps for display and publication—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 12).

SENIOR RESEARCH ASSISTANTS and RESEARCH ASSISTANTS IN FLUIDS MECHANICS—Prof. K. Stewartson, Science Department, The University, South Road, Durham (August 15).

CHAIR OF PATHOLOGY in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

CHAIR OF PHYSICS at Fourah Bay College, University College of Sierra Leone—The Secretary Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 31).

LECTURER (with an honours or higher degree in chemical engineering or equivalent qualifications, and preferably an interest in mass transfer or nuclear chemical engineering) IN CHEMICAL ENGINEERING at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

LECTURERS (3) (with special qualifications in inorganic, organic or physical chemistry) IN CHEMISTRY at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Canterbury (New Zealand and London, August 31).

SCIENTIFIC OFFICER (honours graduate with a special interest in plant breeding and genetics), for work on tree fruits under experienced leadership, as one of a team of fruit breeders—The Secretary, East Malling Research Station, Maidstone, Kent (August 31).

SCIENTIFIC OFFICER (honours graduate), for research on problems arising out of the vegetative propagation of fruit plants—The Secretary, East Malling Research Station, Maidstone, Kent (September 30).

ASSISTANT LECTURER (with a good honours degree, and preferably research and/or industrial experience) IN PHYSICAL CHEMISTRY—The Registrar, Hatfield College of Technology, Hatfield, Herts.

ASSISTANT PROFESSOR (with Ph.D. or equivalent, and preferably with a background and experience in marine biology) OF BIOLOGY, to give instruction primarily in animal physiology—Head, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

CHEMIST (with an honours degree in agricultural chemistry or chemistry, and preferably at least one years postgraduate training at an experimental station) IN THE DEPARTMENT OF AGRICULTURE, Uganda, for research work on soil fertility problems connected with tropical crop rotations, manures and fertilizers—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/9/09/C1.

CHEMIST (with some research experience in physics or biochemistry), to work on certain aspects of the chemistry of wheat protein; and an ASSISTANT (male, with some experience in the management of laboratory animals), for a small animal unit concerned mainly with nutritional research on flour and bread—The Director of Research, Cereals Research Station, Old London Road, St. Albans, Herts.

GRADUATE (preferably with previous experience in tissue culture or virology), to be responsible for the maintenance and supply of tissue cultures needed for research on trachoma and related eye diseases—Dr. L. H. Collier, Trachoma Research Unit, Lister Institute, Chelsea Bridge Road, London, S.W.1.

LECTURER or ASSISTANT LECTURER (with a degree in agriculture, or equivalent, and preferably with some tropical experience) IN AGRICULTURE at the Imperial College of Tropical Agriculture, University College of The West Indies, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

LECTURER (with an honours degree or equivalent qualification in metallurgy or physics) IN THE SCHOOL OF METALLURGY, University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia.

MASTER (with a good honours degree) TO TEACH CHEMISTRY throughout the School—The Headmaster, King Edward VII School, Lytham.

PHYSICISTS, to carry out independent research and to take an interest in a broad range of applications of physics to the building industry—Dr. A. R. Collins, Director of Research and Technical Services, Cement and Concrete Association, Wexham Springs, Stoke Poges, Buckinghamshire.

POSTDOCTORAL RESEARCH FELLOW IN ORGANIC CHEMISTRY—Prof. R. C. Cookson, The University, Southampton.

POSTGRADUATE RESEARCH ASSISTANT (with a knowledge of X-ray diffraction methods), for studies of liquid structure under the personal supervision of Prof. J. D. Bernal—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

RESEARCH ASSISTANT (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield, 10.

RESEARCH ASSISTANT, to help with current research on an immunological project connected with rheumatoid arthritis—The Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

RESEARCH OFFICER (ECONOMIC BOTANIST) (male, between 24–45, with an honours degree in natural science, two years postgraduate training, a good general knowledge of the subject, and preferably some field experience) IN THE DEPARTMENT OF AGRICULTURE, North Borneo, to deal with the selection and breeding of the major crops of North Borneo—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.63/21/09/C1.

RESEARCH TECHNICIAN (with a knowledge of chemistry, physics or biochemistry) to assist in the study of biochemical changes to patients and experimental animals undergoing heart operations by means of heart–lung bypass procedures—The House Governor, Brompton Hospital, South Kensington, London, S.W.7, quoting PR2.

SENIOR LECTURER (qualified to teach microbiology to final degree and Diploma in Technology standard) IN MICROBIOLOGY—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

SENIOR TECHNICIAN (Grade 1) or SCIENTIFIC OFFICER FOR THE MICROBIOLOGICAL ASSAY UNIT (Vitamin B_{12} and folic acid), City

General Hospital—The Group Secretary, Sheffield No. 1 Hospital Management Committee, "Lyndhurst", Nether Edge Hospital, Sheffield 11.

Senior Technician I in the Department of Pathology, for duties confined to experimental pathology—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

REPORTS and other PUBLICATIONS

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